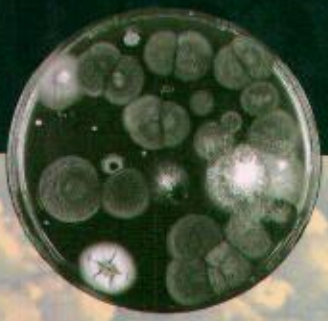

Organic dust exposure and respiratory health in the potato processing industry

Jan-Paul Zock

Stellingen

1. Klachten over longen en luchtwegen tijdens het werk in de aardappelverwerkende industrie zijn voor een belangrijk deel het gevolg van blootstelling aan bacterieel endotoxine.
   Dit proefschrift

2. Acute longfunctieveranderingen bij werknemers van aardappelverwerkende bedrijven zijn gerelateerd aan hun endotoxineblootstelling.
   Dit proefschrift

3. Het hergebruiken van proceswater in aardappelverwerkende bedrijven in het belang van het algemene milieu, heeft geleid tot een verslechtering van het arbeidsmilieu in deze bedrijven.
   Dit proefschrift

4. Werknemers die relatief lang in de aardappelverwerkende industrie werken, hebben een goede respiratoire gezondheid.
   Dit proefschrift

5. In studies naar blootstellings-respons relaties bij ploegendienstwerkers is het belangrijk om alle verschillende diensten in onderzoek te betrekken.
   Dit proefschrift

6. In veel arbeidssituaties kunnen werknemers specifieke IgG antilichamen ontwikkelen tegen stoffen waaraan ze beroepsmatig zijn blootgesteld. Over de gezondheidskundige relevantie van de IgG_4 subklasse is nog weinig duidelijk.

7. De daling van FEV_1 en MMEF over de werkweek bij mengvoederwerkers zoals beschreven door Smid en co-auteurs, kan voor een belangrijk deel worden verklaard door *'effort dependence'* als gevolg van een leereffect.
   Smid T, et al., Am J Ind Med 1994;25:877–88.
   Krowka MJ, et al., Am Rev Respir Dis 1987;136:829–33.
   Hoek G, et al., Eur Respir J 1992;5:553–9.

8. In veel dwarsdoorsnede onderzoeken kan de gehanteerde *Odds Ratio* niet als risicomaat, maar alleen als associatiemaat worden geïnterpreteerd.
   Thompson ML, et al., Occup Environ Med 1998;55:272–7.

9. Het vergelijken van de gezondheidsrisico's van passief roken en peperconsumptie door een grote sigarettenfabrikant is in meerdere opzichten hetzelfde als het vergelijken van de klassieke appels en peren.

10. De spreuk van Jan Pelleboer 'Sneeuw in november, Kerstmis in december' is een fraai voorbeeld van een test met lage sensitiviteit en hoge specificiteit.

11. Het feit dat veel Nederlanders een hekel aan Duitsers hebben, komt omdat Nederlanders en Duitsers veel op elkaar lijken.

12. Aan de vraag om één 'representatief' persoon bij sollicitatieprocedures kan op statistische gronden nooit worden voldaan.

13. Positieve discriminatie brengt per definitie negatieve discriminatie met zich mee.

Stellingen behorende bij het proefschrift
'Organic dust exposure and respiratory health in the potato processing industry'.
Jan-Paul Zock, Wageningen, 9 september 1998.

Organic dust exposure and respiratory health in the potato processing industry

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Organic dust exposure and respiratory health in the potato processing industry

Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit Wageningen,
dr. C.M. Karssen,
in het openbaar te verdedigen
op woensdag 9 september 1998
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"Rabbit's clever," said Pooh thoughtfully.
"Yes," said Piglet, "Rabbit's clever."
"And he has Brain."
"Yes," said Piglet, "Rabbit has Brain."
There was a long silence.
"I suppose," said Pooh, "that that's why he never understands anything."

A.A. Milne
The House at Pooh Corner

Abstract

Organic dust exposure and respiratory health in the potato processing industry

Work-related respiratory health complaints were reported in a potato processing plant. Responsible agents and the underlying mechanism for these disorders were unclear. The first aim of this study was to quantify the occurrence of work-related respiratory health effects in workers of all four plants of the potato processing company. The second aim was to study three possible mechanisms: a Type I allergy to potato, a Type III allergy to microbial components or potato (Hypersensitivity Pneumonitis), and a non-specific airway inflammation caused by microbial components such as bacterial endotoxin.

Sixteen percent of the 142 workers studied indicated work-related respiratory symptoms on a questionnaire. Specific IgE to airborne dust extract could not be detected in workers' sera, and therefore a Type I allergy was highly unlikely to play a role in the etiology of work-related respiratory effects. Specific IgG to airborne dust extract was detected in sera of nearly all workers. This humoral immune response was dominated by the IgG_4 subclass, the latter being present in about half of the workers. The antigens involved appeared to be heat-labile potato proteins. No association was found between specific IgG and the prevalence of work-related respiratory symptoms. On the basis of these findings it was judged improbable that a Type III allergy and with that Hypersensitivity Pneumonitis played a predominant role in the etiology of work-related respiratory effects. High levels of airborne bacteria and endotoxin were found, being significantly related to acute lung function changes. This was observed both for spirometric lung function change across the Afternoon shift, and for peak flow changes across all three types of work shifts. Chronic respiratory effects were not observed in cross-sectional analyses, which might be partially due to a health-related selection in this industry.

Results in this thesis strongly suggest a predominant role of non-specific airway inflammation caused by bacterial endotoxin in the etiology of acute respiratory effects. Recycling of process water contributes to bacterial growth and hence to the build-up of endotoxin. Control measures to reduce endotoxin exposure should be evaluated, with a priority for elimination and/or reduction at the source.

Contents

1. Introduction

Background of the study

Rationale

In the potato processing industry, work-related health complaints have repeatedly been reported. In one of the facilities of a potato processing company, some workers have suffered from respiratory symptoms like shortness of breath and chest tightness, sometimes accompanied by muscular pains and malaise. These symptoms occur mainly during work in the 'fibre dehydration department', containing rolling mills with textile sieves to squeeze water out of organic fibres. In contrast to most other process machinery, the rolling mills constitute an open process, which make aerosolisation of particulates and droplets possible. Warm and humid conditions seem to provide favourable circumstances for flourishing of micro-organisms. Severe symptoms occasionally lead to sick-leave. Most complaints have been reported shortly after the start of the processing campaign (early August), but typically disappear within a few weeks onwards.

In this particular plant, a preliminary survey was conducted and described by Hollander *et al.*[1] This study supported the information available and showed that respiratory symptoms were present in 18 out of 41 production workers; with a rate of 10% for frequent wheezing and 10% for chest tightness. Peak flow patterns suggestive of work-related reversible airflow obstruction were demonstrated. Personal exposure to Gram-negative bacterial endotoxin was up to nearly 500 ng/m^3; job title mean concentrations amounted to 3 to 72 ng/m^3. Specific serum IgG_4 directed against airborne dust extract was found in about half of the workers. Antigenic activity of the airborne dust extract was fully inhibited by potato extract, suggesting that the antigens were most likely potato-derived. Specific IgE to these antigens, however, could not be demonstrated, indicating that a Type I allergy was not likely to play a predominant role. Acute respiratory health outcomes were not measured, and associations between exposure and the outcomes could therefore not be investigated.

After this investigation, the responsible agents and the mechanism of the described disorders in potato processing workers were still unclear. Endotoxin concentrations were in the range where acute respiratory effects can be expected.[2] Furthermore, the specific humoral immune response was limited and only characterised by evaluation of the presence of specific IgG_4 antibodies. The

occurrence of a Hypersensitivity Pneumonitis could therefore not be excluded because other IgG subclasses are involved in the pathology and can act as precipitating antibodies in Type III reactions. Next to potato components, Hypersensitivity Pneumonitis due to exposure to fungi was also possible since Type III reactions to fungal antigens have frequently been described[3], although not in this particular process. Also, the question arose to what extent adverse health effects also occurred in the other three facilities of the company. Finally, only if responsible components and sources of exposure could be identified, it would be possible to develop scientifically based control measures for exposure reduction.

Respiratory health effects in potato workers

A wide range of respiratory health effects in potato workers was found in the literature despite the fact that the available information was limited. Possible mechanisms of respiratory effects were a Type I allergy to potato proteins, a Type III allergy to antigens from micro-organisms associated with potato processing, pneumoconiosis due to respirable silica from soil particles on the potatoes, and non-specific inflammatory reactions due to endotoxin inhalation.

A study in a Polish potato processing plant[4] suggested two possible mechanisms for work-related respiratory effects, which had been characterised by respiratory complaints and across-shift lung function changes. Total airborne counts of micro-organisms were high in this study and amounted to $10^4 - 10^5$ Colony Forming Units per m^3, predominated by the Gram-positive Corynebacteria (2 to 6×10^4 CFU/m^3). *Pseudomonas* spp. and *Acinetobacter* spp. were most common among Gram-negative bacteria (up to ± 500 CFU/m^3). Counts of *actinomycetes* were below 500 CFU/m^3. Concentrations of fungi ranged up to ± 1000 CFU/m^3, dominated by *Aspergillus niger*. Airborne endotoxin concentrations were excessive and ranged up to $10^4 - 10^6$ ng/m^3. These levels were clearly above suggested no-effect levels[2], which ranged from 9 to 180 ng/m^3. Thus, a non-specific inflammation due to these endotoxin levels was one possible mechanism of respiratory effects. A second possible mechanism was a Type III allergy since 67% of the workers had precipitins to microbial antigens deriving from the working environment. The presence of precipitins to certain species was associated with the occurrence of work-related symptoms.

In some occupations where workers are involved in handling potatoes, respiratory effects have also been reported. Greene and Bannan[5] described two cases

of potato riddlers, whose work involved sorting and sieving of potatoes after storage in a pit covered with hay. Both cases showed typical patterns of farmer's lung, known to be associated with exposure to principally *Thermophilic actinomycetes* which grow in mouldy hay.[6] One case even showed progressive pulmonary fibrosis, which has been described in association with farmer's lung.

Jorna *et al.*[7] studied pulmonary fibrosis in a group of 172 potato sorters. The exposure of interest here was diatomaceous earth with a high crystalline silica content. Lung function parameters indicative of obstructive effects (FEV_1, FEV_1/FVC, MMEF and PEF) in exposed workers were significantly lower than in controls. However, no radiological evidence of pneumoconiosis could be detected. Organic dust components were not measured in this study, thus it could not be excluded that obstructive respiratory effects were partly due to dust components from microbial origin.

Also in housewives respiratory health effects of potato handling have been reported. Already in 1966, 29 cases of potato sensitivity were described[8], such as sneezing or wheezing within a few minutes of starting to scrape new potatoes. In this study, six cases showed a considerable reduction in FEV_1 of 17 to 71% during scraping of potatoes for 5 – 10 minutes. A case report from 1967[9] suggested that the potato sensitivity was due to an atopic allergy (Type I) to potato allergens. The allergenic activity had disappeared after heating at 100°C.

Quirce and coworkers[10] described two housewives with raw potato-induced bronchial asthma. A Type I hypersensitivity to raw potato antigens in these cases was demonstrated by means of immediate skin test reactivity, specific IgE determination (RAST), basophil degranulation, histamine release test and an immediate bronchial provocation test response to raw potato extract. After heating potato extract at 75°C for 30 minutes, skin prick testing showed smaller reactions, but was still positive. Two kinds of potato extract which had previously been processed at 100°C, showed negative reactions in skin prick tests. In a laboratory study[11] using electrophoresis, electroblotting and radiography it was demonstrated that specific IgE antibodies in a potato-sensitised subject were directed against several proteins ranging from 14 to 40 kilo-Daltons.

In general, allergic reactions to potato are uncommon.[10,11] However, Bircher *et al.*[12] observed that 29% of 238 patients allergic to one or more of three pollens, had detectable specific IgE to potato allergens. Among 36 patients allergic to cat or house dust mite, and 55 non-atopic individuals in this study specific IgE

against potato was not detected. Profilin, an ubiquitous protein present in eukaryotic cells of animals and plants, has been identified as a major cross-reactive allergen in different pollens, fruits and vegetables.[12] The suggested cross-reactivity between pollen and potato allergens was confirmed in a study among 20 patients with birch pollen allergy.[13] Finally, in one case study it was found that allergy to potato pollen was caused by the same allergens as those in potato tubers.[11]

Respiratory health effects in related industries

Studies in some related sectors of industry show a similar range of respiratory health effects and mechanisms involved. Acute lung function changes, respiratory symptoms and immunological changes in these studies suggested adverse respiratory effects of occupational exposures. A closely related industry in which agricultural crops are processed is sugar beet refinement. An occupational health survey in an English sugar beet processing plant[14] showed mean counts of total airborne bacteria of 2×10^5 CFU/m^3. Levels of Gram-negative bacteria were on average 4,000 CFU per m^3. Predominant bacteria species were common soil-borne bacteria. Airborne fungi and *Thermophilic actinomycetes* occurred only infrequently in the factory air. Endotoxin concentrations ranged from 3 to 30 ng/m^3. Seven of the 13 tested workers had specific serum IgG to beet extract, while five out of 13 had specific IgG to bacterial extracts. Because of the latter finding, it was suggested that Hypersensitivity Pneumonitis was likely to occur, while endotoxin-related respiratory effects were judged unlikely since airborne levels of endotoxin were relatively low.

Most other industries processing agricultural crops have different process technologies, and therefore occupational exposures may be different from potato and sugar beet processing. In many cases, the mechanism of observed respiratory disorders is not fully understood. However, a broad classification into three types of disease entities and underlying responsible mechanisms can be made.

A Type I allergy in these type of occupations is not often reported. Type I allergy to proteins from wheat and rye has been observed among bakery workers[15]. It can be hypothesised that in other occupational settings in which agricultural crops are being processed a Type I allergy might play a role, since food allergy to many agricultural crops has been described[12,13]; among others carrot, soy bean and apple.

A second mechanism is Hypersensitivity Pneumonitis, classically associated with Type III allergy. One of the first occupational diseases in which Type III allergy plays a dominant role is farmer's lung.[6,16,17] This mechanism has later been recognised in many other (mostly agriculture-related) occupations; not only directed against *Thermophilic actinomycetes*, but also against antigens of several genera of fungi, such as in mushroom[3], malt[18] and wood workers[19].

Non-specific airway inflammation is the third mechanism. A well-recognised causative agent in this reaction is bacterial endotoxin[20,21]. Non-specific airway inflammation due to airborne endotoxin exposure has been described extensively in among others cotton[22-24] and other textile workers[22,25,26], grain handlers[27] and animal feed workers[28]. For many occupational environments, endotoxin is one constituent of an heterogeneous organic dust mixture. However, it is believed to comprise a general marker for the presence of (Gram-negative) bacteria in these situations. Other microbial components are also suggested to be inflammatory agents. There is recent interest in peptidoglycan[29,30], a structural cell wall component of (mainly Gram-positive) bacteria, and ß(1→3)-Glucans[31-35], components of fungal or vegetable origin, which also may have inflammatory properties after inhalation.

Definition

The above mentioned literature suggests that different mechanisms can be responsible for work-related respiratory symptoms in potato processing workers. A Type I allergy to potato is uncommon, but can on forehand not be excluded. A Type III allergy is possible since specific IgG reactions to microbial and potato antigens have been observed previously. Finally, non-specific airway inflammation is possible, since high endotoxin exposure levels have been observed, in combination with flu-like symptoms such as malaise and muscular pains.

Aims of the study and outline of the thesis

The main aims of this study were (1) to quantify the occurrence of work-related respiratory health effects in potato processing workers, and (2) to study possible mechanisms. A number of research questions was derived from these aims.

- What are concentration levels, variability and possible determinants of workers' exposure to organic dust constituents?
- What are outcomes of objective acute respiratory health indices?
- Are objective acute respiratory health indices related to organic dust exposure?
- Do work-related allergies, to either potato or microbial components, of Type I or Type III play a role?
- Does a non-specific inflammatory response to organic dust play a role?
- Are chronic respiratory effects of organic dust exposure present?

In order to answer these questions, a comprehensive study in four potato processing plants was set up. The following investigations were made:

- A self-administered questionnaire on occupational history in potato processing, respiratory symptoms, and smoking habits was distributed among all production workers of the four facilities.
- A 'base-line' spirometric lung function test was performed at the start of the processing campaign among all production workers.
- Measurements of exposure to dust, micro-organisms, bacterial endotoxin and airborne antigens were conducted in the four facilities.
- Specific serum IgG and IgE antibodies against airborne dust extract were measured in blood samples taken before, as well as during the campaign.
- Total serum IgE and specific IgE antibodies against common allergens were measured in blood samples taken before, as well as during the campaign.
- Repeated spirometric lung function tests before and after the work shift were performed among all production workers in one of the four facilities.
- Repeated peak flow measurements during different work shifts and leisure days were performed by all production workers.

In Chapter 2 an overview is given of exposure measurements of dust, endotoxin and micro-organisms in the four potato processing plants. Determinants of exposure are discussed, and it is described how workers can be grouped with regard to their personal dust and endotoxin exposure. Chapter 3 gives the results of an experimental study of filter elution methods for measurement of airborne antigens. In Chapter 4 the specific IgG response in relation to airborne antigen exposure is described. Chapter 5 presents the results of a study on acute lung function changes in relation to relatively low endotoxin exposures in one of the plants. In Chapter 6 acute peak flow changes across the three different work shifts are described, in relation to endotoxin exposure. In Chapter 7 possible chronic effects of organic dust exposure are investigated in a cross-sectional analysis. Finally, in the general discussion in Chapter 8 main findings and answers to the research questions are discussed. Methodological issues, potential biases, implications and need for further study are given attention. The most important conclusions of this thesis are summarised.

References

1. Hollander A, Heederik D, Kauffman H. Acute respiratory effects in the potato processing industry due to a bio-aerosol exposure. *Occup Environ Med* 1994; 51: 73–78.
2. Douwes J, Heederik D. Epidemiologic investigations of endotoxins. *Int J Occup Environ Health* 1997; 3: S26–S31.
3. Kurup VP, Barboriak JJ, Fink JN. Hypersensitivity Pneumonitis. In: Al-Doory Y, Domsan JF (eds). Mould Allergy. Philadelphia: Lea & Febiger, 1984: 216–243.
4. Dutkiewicz J. Bacteria, fungi and endotoxin as potential agents of occupational hazard in a potato processing plant. *Am J Ind Med* 1994; 25: 43–46.
5. Greene JJ, Bannan LT. Potato riddler's lung. *Irish Med J* 1985; 78: 282–284.
6. Marx JJ, Emanuel DA, Dovenbarger WV, Reinecke ME, Roberts RC, Treuhaft MW. Farmer's lung disease among farmers with precipitating antibodies to the thermophilic actinomycetes: A clinical and immunologic study. *J Allergy Clin Immunol* 1978; 62: 185–189.
7. Jorna THJM, Borm PJA, Koiter KD, Slangen JJM, Henderson PTh, Wouters EFM. Respiratory effects and serum type III procollagen in potato sorters exposed to diatomaceous earth. *Int Arch Occup Environ Health* 1994; 66: 217–222.
8. Bruce Pearson RS. Potato sensitivity, an occupational allergy in housewives. *Acta Allergologica* 1966; 21: 507–514.
9. Nater JP, Zwartz JA. Atopic allergic reactions due to raw potato. *J Allergy* 1967; 40: 202–206.

10. Quirce S, Díez Gómez ML, Hinojosa M, Cuevas M, Ureña V, Rivas MF, Puyana J, Cuesta J, Losada E. Housewives with raw potato-induced bronchial asthma. *Allergy* 1989; 44: 532–536.
11. Castells MC, Pascual C, Martin Esteban M, Ojeda JA. Allergy to white potato. *J Allergy Clin Immunol* 1986; 78: 1110–1114.
12. Bircher AJ, Van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. *Clin Exp Allergy* 1994; 24: 367–374.
13. Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, Kraft D, Scheiner O. Identification of allegens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens *Bet v* 1 and *Bet v* 2 (birch profilin). *J Allergy Clin Immunol* 1995; 95: 962–969.
14. Forster HW, Crook B, Platts BW, Lacey J, Topping MD. Investigation of organic aerosols generated during sugar beet slicing. *Am Ind Hyg Assoc J* 1989; 50: 44–50.
15. Houba R. Occupational respiratory allergy in bakery workers: Relationships with wheat and fungal α-amylase aeroallergen exposure. Thesis. Wageningen Agricultural University, Wageningen, 1996.
16. Cormier Y, Laviolette M. Farmer's lung. *Semin Respir Med* 1993; 14: 32–37.
17. Roitt I. Essential Immunology. Eighth edition. Cambridge MA: Blackwell Scientific Publications, 1994: 326–331.
18. Christiani DC. Organic dust exposure and chronic airway disease. *Am J Respir Crit Care Med* 1996; 154: 833–834.
19. Eduard W, Sandven P, Levy F. Exposure and IgG antibodies to mould spores in wood trimmers: Exposure-response relationships with respiratory symptoms. *Appl Occup Environ Hyg* 1994; 9: 44–48.
20. Castellan RM, Olenchock SA, Kinsley KB, Hankinson JL. Inhaled endotoxin and decreased spirometric values. An exposure-response relation for cotton dust. *N Engl J Med* 1987; 317: 605–610.
21. Rylander R, Bake B, Fischer JJ, Helander IM. Pulmonary function and symptoms after inhalation of endotoxin. *Am Rev Respir Dis* 1989; 140: 981–986.
22. Sigsgaard T, Pedersen OF, Juul S, Gravesen S. Respiratory disorders and atopy in cotton, wool and other textile mill workers in Denmark. *Am J Ind Med* 1992; 22: 163–184.
23. Rylander R, Haglind P, Lundholm M. Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. *Am Rev Respir Dis* 1985; 131: 209–213.
24. Kennedy SM, Christiani DC, Eisen EA, Wegman DH, Greaves IA, Olenchock SA, Ting-Ting Y, Pei-Lian L. Cotton dust and endotoxin exposure-response relationships in cotton textile workers. *Am Rev Respir Dis* 1987; 135: 194–200.
25. Zuskin E, Kanceljak B, Mustajbegovic J, Godnic-Cvar J, Schachter EN. Immunological reactions and respiratory function in wool textile workers. *Am J Ind Med* 1995; 26: 445–456.
26. Buick JB, Lowry RC, Magee TRA. Isolation, enumeration, and identification of Gram-negative bacteria from flax dust with reference to endotoxin concentration. *Am Ind Hyg Assoc J* 1994; 55: 59–61.
27. Schwartz DA, Thorne PS, Yagla SJ, Burmeister LF, Olenchock SA, Watt JL, Quinn TJ. The role of endotoxin in grain dust-induced lung disease. *Am J Respir Crit Care Med* 1995; 152: 603–608.

28. Smid T, Heederik D, Houba R, Quanjer PhH. Dust- and endotoxin-related respiratory effects in the animal feed industry. *Am Rev Respir Dis* 1992; 146: 1474 – 1479.
29. Verhoef J, Kalter E. Endotoxic effects of peptidoglycan. In: ten Cate JW, Büller HR, Sturk A (eds). Bacterial endotoxins: Structure, biomedical significance, and detection with the Limulus Amebocyte Lysate test. New York: Alan R. Liss Inc, 1985: 101 – 112.
30. Burroughs M, Rozdzinski E, Geelen S, Tuomanen E. A structure-activity relationship for induction of meningeal inflammation by muramyl peptides. *J Clin Invest* 1993; 92: 297 – 302.
31. De Lucca AJ, Brogden KA, French AD. Agglutination of lung surfactant with glucan. *Br J Ind Med* 1992; 49: 755 – 760.
32. Fogelmark B, Sjöstrand M, Rylander R. Pulmonary inflammation induced by repeated inhalations of ß(1→3)-D-glucan and endotoxin. *Int J Exp Pathol* 1994; 75: 85 – 90.
33. Rylander R, Persson K, Goto H, Yuasa K, Tanaka S. Airborne beta-1,3-glucan may be related to symptoms in sick buildings. *Indoor Environ* 1992; 1: 263 – 267.
34. Williams DL. (1→3)-ß-D-glucans. In: Rylander R, Jacobs R (eds). Organic dusts: exposure, effects, and prevention. Chicago: Lewis Publishers, 1994: 83 – 85.
35. Douwes J, Doekes G, Montijn R, Heederik D, Brunekreef B. Measurement of ß(1→3)-Glucans in occupational and home environments with an inhibition enzyme immunoassay. *Appl Environ Microbiol* 1996; 62: 3176 – 3182.

2. Exposure to dust, endotoxin and micro-organisms in the potato processing industry[1]

Abstract

Exposure to organic dust components was studied in four potato processing plants because preliminary results showed high exposures accompanied by work-related health complaints. Ambient air concentrations of inhalable dust ranged from below 0.4 up to 44 mg/m^3 (Geometric Mean (GM) 0.64 mg/m^3). Respirable dust concentrations were considerably lower. Personal concentrations of inhalable dust were somewhat higher, and strongly related to a few working tasks dealing with dried starch or protein. Ambient air concentrations of endotoxin ranged from 0.5 to more than 60,000 Endotoxin Units (EU) per m^3 for the inhalable size fraction (GM = 280 EU/m^3). For the respirable size fraction, endotoxin concentrations were lower (about the same factor as for dust). Personal endotoxin concentrations were lower than ambient air concentrations, probably because workers did not work the whole period of the shift near endotoxin sources. Endotoxin exposure was evaluated as very high; 23% of the workers had a mean exposure above 1000 EU/m^3 (100 ng/m^3). Differences between plants had a large influence on both dust and endotoxin exposure. A fairly good correlation was found between counts of airborne Gram-negative bacteria and airborne endotoxin of the respirable size fraction. Ambient air levels of bacteria and endotoxin were strongly related to process water temperature, suggesting that exposure reduction can be achieved by lowering this temperature or by other measures that inhibit bacterial growth. We conclude that recycling of process water probably constitutes an exposure source of bacteria and endotoxin in many facilities.

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[1] Jan-Paul Zock, Dick Heederik, Hans Kromhout.
The Annals of Occupational Hygiene 1995; 39: 841 – 854.

Introduction

In north-western Europe special potatoes are grown for industrial starch production. In potato processing companies, potatoes are processed into starch and co-products such as protein, fibres and protamylasse (raw materials for animal food production).

An earlier study on occupational exposure to organic dust components in a potato processing plant was performed and reported by Hollander *et al.*[1] Results showed very high concentrations of bacteria and endotoxin and a high prevalence of work-related respiratory symptoms. Sources of exposure remained unclear, and therefore auxiliary data from similar facilities were collected in a second study.

This study was conducted to achieve an overview of exposure to organic dust in all four plants of the company. Results were meant to be used in a larger study on respiratory effects in potato processing workers. Besides, analyses of exposures in relation to the different process techniques in the four plants were used to draft a control strategy for departments with high exposure to organic dust.

The production process consists of relatively simple, though highly mechanised physical techniques. Potatoes are delivered by truck, weighed, tested and stored. The first step is washing the potatoes to remove dirt, leaves and stones. Subsequently, potatoes are ground and separated in fruit water (potato juice with protein and other dissolved components) and solid components, either by centrifugation or by hydrocyclones. The fruit water is concentrated and after denaturation (heating and acid addition) the protein is separated, dried and shipped. The rest of the fruit water is evaporated into protamylasse, a syrup to be used in animal food production. The solid components are separated into starch and fibres by centrifuging. Starch is refined by centrifuging / sieving, dried and sacked in big bags or shipped into silos. Fibres are dehydrated by either centrifugation or by squeezing in a rolling mill (only plant 2).

The plants are in continuous operation in the period August–March (shortly after the start of the potato harvest), referred to as 'campaign'. Differences in processing capacity between plants are large, ranging from 90 to 230 tons of potatoes per hour. Employees work in five shifts, each shift in each plant consists of 13 workers. The main task of most workers consists of process control,

including taking process samples. Exposure to organic dust mainly takes place during cleaning, sampling, maintenance, controlling technical failures, and during activities in expedition departments.

Extremely large amounts of water are used in different steps of the process. For environmental and financial reasons, most water is recycled. Each facility has its own sewage treatment plant. Warm and humid conditions and the water recycling provide good opportunities for growth of micro-organisms.

In this study, exposure levels of several components of organic dust were determined in all four plants. Ambient air as well as personal monitoring were carried out. Special attention was paid to endotoxin, a cellular component of Gram-negative bacteria, for which adverse health effects have been reported.[2,3] Next to a description of exposures, the study focused also on determinants of exposure to characterize sources of exposure, that would be useful for the development of control strategies.

Material and methods

Measurements were carried out in the autumn of 1992. Each plant was studied for 10 days, to obtain repeated personal exposure data from two consecutive days of workers within each Morning shift, and ambient air concentrations of all relevant departments.

Ambient air sampling

Ambient air sampling was conducted in departments where worker's activities were concentrated. Dust was sampled on two successive days on the same spot. Two different particle size fractions were determined: inhalable and respirable dust. Three different groups of viable micro-organisms were determined: fungi, bacteria (referred to as 'total-bacteria') and Gram-negative bacteria. For practical reasons, all samples of micro-organisms in a plant were taken on the same day. All measurements were done during the Morning shift (7 a.m. to 3 p.m. or 6 a.m. to 2 p.m.).

Inhalable dust was determined using portable constant-flow sampling pumps (Dupont P-2500 and Gilian Gil-Air) at a flow rate of 2.0 L/min in combination with PAS 6 sampling heads[4] and Whatman GF/A glass fibre filters with a diameter of 25 mm. The pumps were adjusted with a flow meter at 2.0 L/min before sampling. After sampling, the flow was measured with the same flow meter. The acceptable range for the mean flow was set between 1.8 and 2.2 L/min. Inhalable dust samples were taken in duplicate.

On the same days and spots, respirable dust was sampled. This size fraction was determined using Becker VT6/VT3.6 pumps with gas meters at a flow rate of 50 L/min in combination with cyclones as described by Vrins and Hofschreuder[5] and Whatman GF/A glass fibre filters with a diameter of 47 mm. This results in a 50% cutoff diameter (D_{50}) of 3.6 μm. The acceptable range for the mean flow was set between 40 and 60 L/min; the expected D_{50} is then between 3.2 and 4.3 μm.[5] For practical reasons, only a few duplicate measurements could be taken.

The minimum dust sampling time was set at 6 hours. All measurements were taken at approximately 1.5 meter above ground level with the inlet pointed downward. For respirable dust sampling the inlets of the cyclones pointed sideward. All samples were analyzed gravimetrically in a conditioned weighing room at a temperature of 20 ± 2 °C and 50 ± 5 % relative humidity. After the measurements, filters were stored at −20°C.

Extracts of dust samples were made in order to determine endotoxin levels. One out of two daily duplicate samples of inhalable dust within each department and one out of two samples of respirable dust within each department was extracted with 5 mL (25 mm filters) or 10 mL (47 mm filters) pyrogen-free water (NPBI, Emmer-Compascuum, The Netherlands) with 0.05 % v/v Tween-20 (Merck, Schuchardt, Germany). Using the Tween-addition, the extraction efficiency was seven times better than using pyrogen-free water only.[6] Endotoxin was analyzed in the samples using a kinetic modification of the Limulus Amoebocyte Lysate (LAL) assay as described by Hollander *et al.*[7]. Because batches of samples were analyzed using different standard endotoxins, two conversion factors had to be used to convert the outcome of the test (in ng/mL) to Endotoxin Units (EU/mL), relative to the US endotoxin standard EC-5 (in this case 8.0 and 13.5 EU/ng).

Airborne micro-organisms were sampled using the N-6 modification of the Andersen sampler[8] at a flow rate of 28.3 L/min and a sampling time of 1 minute. Three different media (Oxoid, Hampshire, England) were used: Dichlorane Glycerol Agar (DG18) for fungi[9], Tryptone Soy Agar for total-bacteria and Plate Count Agar with 1 mg/L Crystal Violet (Merck, Darmstadt, Germany) for Gram-negative bacteria. All samples were taken in duplicate at ± 1.5 meter above ground level with the inlet pointed downward. After sampling, agar plates were incubated for four days at 24–25°C (fungi) or for about 24 hours at 37°C (bacteria). After incubation colonies were counted, and corrected for multiple hits as described by Andersen[10]. Concentration was expressed in Colony Forming Units per m^3 (CFU/m^3). Results from agar plates that were overgrown by fast-growing colonies, as well as extremely dry or damp plates, were not used in analyses (five plates = 1.5%). In all departments, temperature and relative humidity were measured real-time for a 7-day period.

Personal sampling

Personal samples were taken during the morning shift. Inhalable dust was sampled during full-shift periods using the same techniques as described earlier. All filters arising from the personal measurements were cut in two using a sterile razor blade. All halves were weighed again, and extracted for either endotoxin analysis, or for antigen analysis. Antigen analysis will be discussed in chapter 3/4.

Data on work characteristics in present and past were obtained by a self-administered questionnaire. Workers were grouped in categories based on job title. Twelve job categories were made comprising one or more jobs with similar tasks.

Statistical analysis

Detection limits were calculated by adding the mean plus three times the standard deviation of 30 blanks (inhalable dust) or 15 blanks (respirable dust), and dividing it by the mean sampled volume.[11] In statistical analyses, values below the detection limits were given a random value between zero and the detection limit using the distribution of concentrations above the detection limit, as proposed by Helsel[12].

The reproducibility was evaluated by computing the mean coefficient of variation (CV) of a series of duplicate measurements with both concentrations above the detection limit. Arithmetic means of duplicates were used in statistical analyses.

Within- and between-worker variances were calculated using random effects analyses of variance[13]. The ratio of 97.5th and 2.5th percentiles of the between-worker distribution (${}_{BW}\hat{R}_{.95}$) was calculated for groups with more than 10 measurements as proposed by Rappaport[14].

Specific measurements in relation to meteorological conditions

In the fibre dehydration department of plant 2 (containing 6 rolling mills) specific measurements were done in the summer and autumn of 1993 to characterize the pattern in airborne concentrations in relation to climatic changes. From the results of the 1992 survey it became clear that airborne concentrations of bacteria were too high to sample directly with the N-6 modification of the Andersen sampler for 1 minute (upper detection limit $\pm 10^5$ CFU/m^3). Even when the N-6 was used at a flow rate of 1 L/min for 15 seconds, agar plates were overloaded (>400 colonies). As an estimation of the airborne concentration bacteria, $>10^7$ CFU/m^3 was taken (>2500 CFU using multiple hit correction in 0.25 litres of air). Air sampling was therefore also done using two types of liquid impingers with 50 or 10 mL sterilized physiological saline (0.85 % w/v NaCl) for 30 or 5 minutes, respectively, at a flow rate of 1 L/min.[15] After sampling, 200 μL of the solution was plated in duplicate on the three different media. Plates were incubated as described before, and airborne concentrations were estimated. Airborne inhalable endotoxin concentrations were determined as described before. Temperature of process water, ambient temperature and relative humidity were measured on the sampling days.

Results

Ambient air measurements

One hundred and thirty-two inhalable dust measurements were available for statistical analyses, including 51 duplicates. The detection limit amounted to 0.41 mg/m^3; 66 measurements (50%) were below this value and given a dis-

tribution-based random value between 0 and 0.41 mg/m^3. The mean coefficient of variation (CV) was 18.6% (N=21; range 0 to 68%). A number of 82 respirable dust concentrations was successfully determined, including 4 duplicates. The detection limit was 0.061 mg/m^3; 23 measurements (28%) were below this value and given a distribution-based random value between 0 and 0.061 mg/m^3. The mean CV was 21% (N=4; range 5.2 to 62%). Extracts were made of 68 inhalable dust samples and 47 respirable dust samples for endotoxin analysis. The mean CV of endotoxin analysis calculated with 340 duplicates was 28% (range 0–172%). Three duplicate airborne concentrations of respirable endotoxin were available, the CV ranged 5 to 10%. The detection limit for endotoxin analysis was 0.2 EU/mL. Samples below this level, 19 for inhalable endotoxin (3%) and 3 for respirable endotoxin (3%), were set at 0.1 EU/mL. Mean CV values for micro-organisms amounted to 28% for fungi, 16% for total-bacteria and 40% for Gram-negative bacteria (total range 0 to 141%).

Distributions of all concentrations appeared to be skewed to the right, therefore statistical analyses were performed using log-transformed concentrations.

Table 2.1: Descriptive statistics of ambient concentrations of dust, endotoxin and micro-organisms

	N	AM	GM	GSD	Range
Inhalable dust (mg/m^3)	81	2.15	0.64	3.9	<0.4 – 44.2
Respirable dust (mg/m^3)	78	0.18	0.11	2.7	<0.06 – 1.58
Inhalable endotoxin (EU/m^3)	68	1879	279	6.1	0.5 – 62,227
Respirable endotoxin (EU/m^3)	47	169	44	5.9	1.7 – 1420
Fungi (10^3 CFU/m^3)	38	1.6	1.0	2.3	0.2 – 15.1
Total bacteria (10^3 CFU/m^3)	38	18.5	6.7	7.1	0.1 – 94.8
Gram-negative bacteria (10^3 CFU/m^3)	39	7.4	1.2	13	0 – 29.3

Descriptive statistics of all ambient air concentrations are summarized in Table 2.1. The variability in concentration appeared to be relatively high, as indicated by the high GSD's. Only for fungi the GSD was below 3 and was reasonably uniform across departments. Dust levels above 10 mg/m^3 were found in expedi-

tion departments where starch or protein was sacked in big bags of 800 and 1000 kgs respectively. The highest endotoxin levels, 25,000 EU/m^3 (~1900 ng/m^3) and 62,000 EU/m^3 (~4600 ng/m^3) were found in the fibre dehydration department of plant 2, which has open rolling mills with textile bands for dehydrating fibres (instead of closed centrifuges in the three other plants).

Both dust and endotoxin levels were related to particle size. The respirable dust fraction contributed only for a relatively small part to the inhalable dust concentrations. For each department, the proportion of respirable dust as percentage of inhalable dust was calculated. The average proportion was 15%. For departments with a high starch or protein dust concentration, the proportion was 2% and 4%, respectively. This indicates that airborne dust in these departments consisted of relatively large particles.

Table 2.2: Correlation coefficients (Pearson's *R*) between log-transformed mean concentrations of dust, endotoxin and micro-organisms; *N* ranges from 35 to 43.

	Respirable dust	Inhalable endotoxin	Respirable endotoxin	Fungi	Total bacteria	Gram-negative bacteria
Inhalable dust	+0.44†	−0.09	−0.15	−0.02	−0.44†	−0.23
Respirable dust		+0.04	+0.23	+0.12	+0.37*	+0.42*
Inhalable endotoxin			+0.83‡	+0.03	+0.47†	+0.60‡
Respirable endotoxin				+0.01	+0.67‡	+0.74‡
Fungi					+0.06	+0.20
Total bacteria						+0.90‡

* $P<0.05$ † $P<0.01$ ‡ $P<0.001$

Table 2.2 shows the correlation between average concentrations for each department of each plant for all exposure indices. The Table shows some interesting findings. Inhalable dust level was weakly and negatively correlated with bacterial counts, whereas respirable dust level was significantly positively correlated with bacterial counts. This correlation was predominantly determined by the departments with a low relative humidity (and a high dust concentration). Correlations between bacterial counts and endotoxin levels were positive for both dust fractions, but higher for the respirable fraction. In Fig. 2.1 a scatterplot is shown of respirable endotoxin concentrations versus Gram-negative

bacteria concentrations. Roughly half the variability in (log) endotoxin concentration was explained by the variability in (log) bacteria concentration ($R^2 = 0.74^2$).

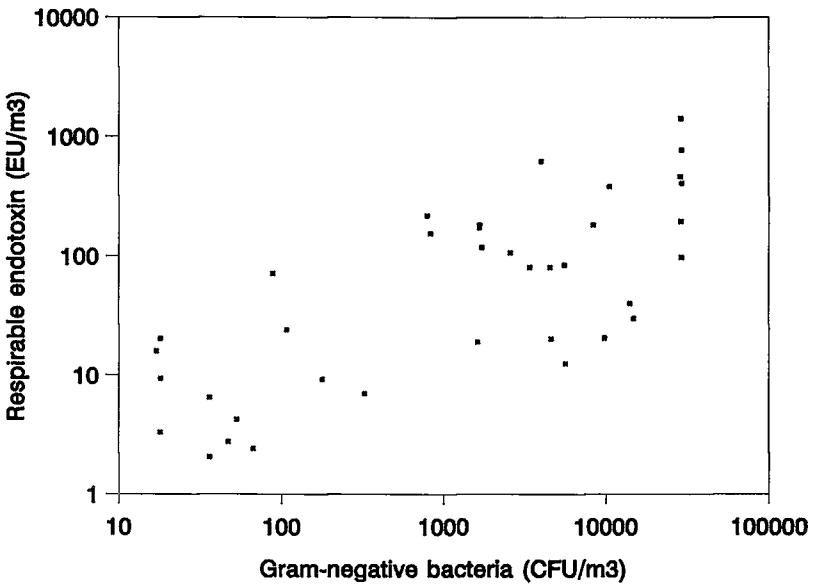

Fig. 2.1: Scatterplot of ambient air respirable endotoxin concentrations versus Gram-negative bacteria concentrations; $N = 37$ and Pearson's R (calculated with log-transformed concentrations) = 0.74.

In Table 2.3 the geometric mean endotoxin content of the sampled dust with 95% confidence intervals is shown for each plant. Analysis of variance (random effects model) showed that the within-plant variations (72% of total variance) were much larger than the between-plant variations (16%), probably due to differences in process technology and climate between measurement sites.

However, large differences between plants can be seen in Table 2.3; a factor 10 to 20-fold between plant 2 and 3 in GM was found. The endotoxin content of inhalable dust was about the same as found for respirable dust. Particles from relatively humid departments had a higher endotoxin content than particles from relatively dry departments (results not shown). When departments were dichotomized in 'dry' and 'humid' using a cut-off level of 55% relative humidity (median level of all measurements), the geometric mean of the endotoxin con-

tent of inhalable dust was 363 and 1017 EU/mg, respectively (difference not statistically significant). For respirable dust, geometric means were 251 EU/mg for dry departments and 805 EU/mg for humid departments (Student's *t*-test, $P<0.05$).

Table 2.3: Endotoxin content (EU/mg) of ambient air dust samples in four plants

	Inhalable dust			Respirable dust		
Plant	*N*	GM	CI*	*N*	GM	CI
1	21	316	109 – 918	13	276	82 – 925
2	18	1986	631 – 6246	12	1084	412 – 2852
3	17	105	43 – 254	12	137	73 – 255
4	16	862	206 – 3604	10	936	293 – 2992

* 95% confidence interval of geometric mean

Personal measurements

A total of 211 personal dust measurements from 128 workers and 195 endotoxin measurements from 123 workers was available for statistical analyses. Repeated dust measurements were available from 83 workers, while 72 workers had repeated endotoxin measurements. The detection limit for dust was 0.40 mg/m^3; 40 measurements (19%) below this level were given a distribution-based random value between 0 and 0.40 mg/m^3. (For detection limits for endotoxin, see under ambient air measurements.) Concentrations appeared to be skewed to the right; analyses were performed with log-transformed values. Pearson's correlation coefficient between log-transformed personal concentrations of dust and endotoxin was only 0.03.

In order to investigate to which extent exposure levels were determined by characteristics of 'plant' and 'job category', analyses of variance (fixed effects model) were performed on log-transformed dust and endotoxin levels. Using one-way analyses of variance, 'job category' explained more variance than 'plant', for both dust and endotoxin. This means that differences in exposure between plants were larger than differences between jobs. In multiple models, the factors

'plant' and 'job category' significantly contributed to the total variance in exposure, for both dust and endotoxin. Inclusion of an interaction term in the analysis showed an increase in explained variance, probably due to the fact that not every job was carried out in a similar way in every plant.

In Tables 2.4 and 2.5 descriptive statistics of personal exposure in 12 job categories and all four plants are given for dust and endotoxin, respectively.

Table 2.4: Descriptive statistics of personal dust concentrations (mg/m^3) by job category and by plant

Job category / plant	*N*	*K*	AM	GM	GSD	$_{BW}\hat{R}_{.95}$*	Range
Technicians	20	11	1.8	1.1	3.0	20	<0.4 – 9.6
All-round	55	33	4.3	0.9	4.0	32	<0.4 – 153
Starch unit	20	12	0.6	0.3	4.0	1.0	<0.4 – 3.5
Co-products unit	24	16	3.2	1.4	4.5	161	<0.4 – 17.1
Unloading/prewash	7	5	0.7	0.6	3.1	—	<0.4 – 1.1
Washing department	23	14	1.2	0.6	3.4	55	<0.4 – 8.3
Grinding department	9	5	0.7	0.5	2.2	—	<0.4 – 0.9
Extraction/refinery	18	12	1.3	0.9	2.0	2.2	<0.4 – 6.6
Drying departments	13	7	4.6	3.2	2.5	29	0.8 – 16.9
Fibre dehydration	8	5	0.5	0.4	3.4	—	<0.4 – 0.9
Starch sackers	11	6	10.8	3.6	5.2	446	0.6 – 42.8
Protein sackers	3	2	29.0	21.1	3.2	—	7.5 – 57.9
Plant 1	30	19	0.8	0.6	2.4	4.4	<0.4 – 1.9
Plant 2	31	20	4.3	1.2	5.0	4.9	<0.4 – 57.9
Plant 3	71	43	3.9	1.8	3.2	57	<0.4 – 42.8
Plant 4	79	46	3.2	0.6	4.1	31	<0.4 – 153
All	211	128	3.3	1.0	4.0	62	<0.4 – 153

* Ratio of 97.5th and 2.5th percentiles of the between-worker distribution
— Not calculated because numbers were too small

Dust exposures were relatively low, except for sackers and operators of the drying departments. Probabilities for non-compliance with the Dutch Occupational Exposure Standard (OES) for total nuisance dust of 10 mg/m^3 (8 hour TWA) were respectively 74% for protein sackers, 27% for starch sackers and 11% for operators of drying departments. This will probably be an underestimation, because the inhalable size fraction instead of total dust was measured in this study.

Table 2.5: Descriptive statistics of personal endotoxin concentrations (EU/m^3) by job category and by plant

Job category / plant	*N*	*K*	AM	GM	GSD	$_{BW}\hat{R}_{.95}$*	Range
Technicians	19	11	156	90	2.8	8.4	22 – 948
All-round	51	31	909	262	4.5	188	28 – 13780
Starch unit	20	12	415	290	2.5	5.3	44 – 1439
Co-products unit	22	16	289	104	3.6	62	14 – 2971
Unloading/prewash	5	4	1131	349	4.8	—	144 – 4914
Washing department	23	14	1531	770	4.2	111	32 – 4675
Grinding department	7	4	562	489	1.8	—	262 – 1070
Extraction/refinery	15	11	1519	860	4.0	153	42 – 4101
Drying departments	13	7	341	175	3.3	38	37 – 1717
Fibre dehydration	8	5	8167	1016	19	—	43 – 29083
Starch sackers	10	6	390	120	6.8	1616	10 – 1442
Protein sackers	2	2	239	192	2.7	—	95 – 383
Plant 1	23	16	239	83	3.6	91	14 – 2971
Plant 2	30	20	3352	678	6.3	811	43 – 29083
Plant 3	66	42	735	290	4.1	114	26 – 4914
Plant 4	76	45	673	256	4.3	127	10 – 4675
All	195	123	1055	272	4.8	232	10 – 29083

* Ratio of 97.5th and 2.5th percentiles of the between-worker distribution
— Not calculated because numbers were too small

Endotoxin exposure was in general very high. A suggested threshold level for adverse health effects is 9 ng/m^3 (about 90 EU/m^3); 77% of the workers had a mean exposure above 100 EU/m^3. The highest endotoxin exposures of 18,000 EU/m^3 (~1300 ng/m^3) and 29,000 EU/m^3 (~2200 ng/m^3) were found for operators of the fibre dehydration department of plant 2. Variation in endotoxin exposure was extremely high; even within job categories GSD's were larger than 3. The between-worker distributions showed that in none of the job categories workers were uniformly exposed to endotoxin; ratios of 97.5th and 2.5th percentiles of the between-worker distributions ($_{BW}\hat{R}_{.95}$) well exceeded 4. For dust, some categories constituted uniformly exposed groups. The within-worker variances differed between job categories for both dust and endotoxin; GSD's ranged from 1.1 to 3.2 (results not shown).

The endotoxin content of personal sampled dust was on average (GM = 257 EU/mg) two times lower than the endotoxin content of ambient sampled inhalable dust. Large differences in mean endotoxin content were found between the different job categories. Operators from the fibre dehydration department (mainly plant 2) were exposed to particles with the highest endotoxin content (GM = 2000 EU/mg). Starch and protein sackers were exposed to dust with the lowest endotoxin content (GM = 30 and 15 EU/mg, respectively).

Specific measurements in relation to meteorological conditions

In Fig. 2.2, ambient air concentrations of micro-organisms and endotoxin on different days at the fibre dehydration department of plant 2 are presented. On day 0, just before the start of the campaign, no potatoes were processed. On days 1 and 2, only water was used in the process to trim machinery. At point A, actual potato processing started. At days 7 and 8, outside ambient temperatures were 15 to 20°C. Point B indicates a change in outdoor temperature; at days 22 and 23 outside ambient temperatures had increased to 20 to 25°C (end of August). Point C indicates another climate change; days 85, 86 and 87 were in the beginning of November, after a relative cold period with night-frost.

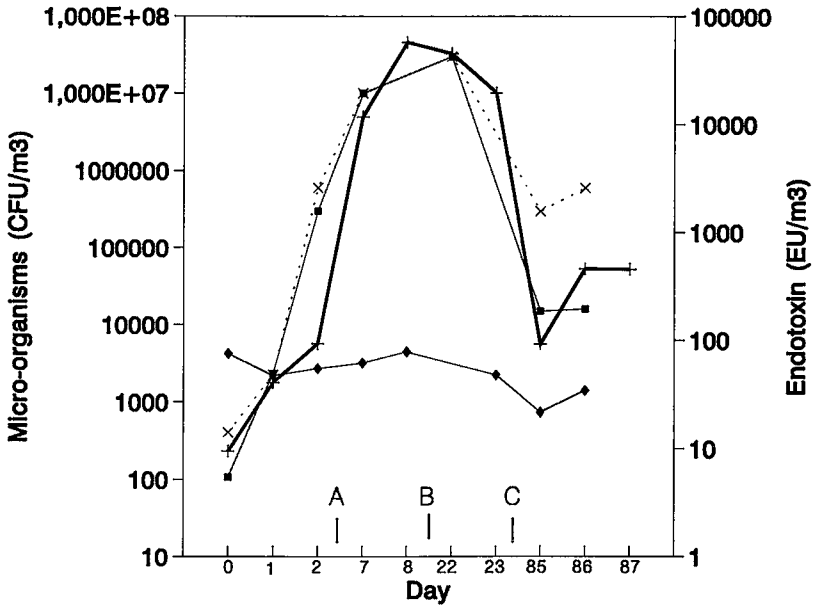

Fig. 2.2: Pattern in fungi (♦), total-bacteria (×), Gram-negative bacteria (■), and endotoxin (+) concentrations in the fibre dehydration department of plant 2 over a period of 87 days since start of the campaign. Point A indicates start of potato processing, B a change into high ambient temperatures and C a change into low ambient temperatures.

From Fig. 2.2 it becomes clear that after the start of processing potatoes, bacterial concentrations rapidly increased. Endotoxin concentrations increased just a little later. Later on, in autumn, exposure decreases again dramatically (about 100-fold). Process water temperature was 24°C at day 2, 30°C at day 7 and 21°C at day 86. The concentration of airborne fungi was not affected by changes in temperature.

Discussion

In this study, indices of organic dust levels were measured in four potato processing plants. The reproducibilities of sampling methods were in compliance with former studies using the same techniques.[7,16] Plants differed in size and process technology, and appeared to explain a great part of the differences in exposure levels. Dust levels were generally low, but operators working with the final (dried) products starch and protein, were exposed to high dust concentrations and had a fair chance for exceeding the OES of 10 mg/m^3. The dust consisted mainly of larger particles, therefore the OES for respirable dust (5 mg/m^3) was probably not exceeded. Most departments with humid conditions showed low dust concentrations close to or under the detection limit. Personal dust concentrations were in general higher than ambient air dust concentrations. This may be due to the fact that operators in dusty departments work closer to dust sources like sacking machines than the locations of ambient air sampling.

Endotoxin is an important constituent of organic dust, which provides a more stable and more relevant index than using viable techniques.[17] Levels were very high, accompanied by high airborne bacterial concentrations ($>10^5$ CFU/m^3). Sources can be found in the humid and warm process, and recirculation of waste water will support microbiological growth. Ambient air endotoxin levels were higher than personal levels, suggesting that endotoxin exposure is more determined by the working environment than by working practices, as opposed to dust exposure.

In an earlier study[1] of plant 2, measured dust exposure levels (GM ranged from 0.5 to 56 mg/m^3) were similar to our findings. Measured endotoxin levels in our study were however roughly ten times higher then reported by Hollander[1] (GM ranged from 3.4 to 72 ng/m^3). This is probably caused by the Tween-addition during extraction procedures.[6]

A study in a potato processing plant in Poland[18] with a different process (potatoes are peeled and subsequently steamed), showed higher endotoxin concentrations in departments ensuing the steaming procedures (up to 1 mg/m^3). However, results can hardly be compared because of different measurement and analytical techniques.

Both for dust and for endotoxin it was found that personal exposures differed strongly between plants. For dust this can be explained by different shipping

procedures; two plants used mostly bags for storage (mainly handwork); the other two used only conveyer belts to silos (mainly mechanized). For endotoxin, the stated differences can be due to the large differences in endotoxin content of the measured particles. This can partly be explained by differences in water recycling, which causes large differences in bacterial growth and consequently building up of endotoxin levels.

Rough indices such as gravimetric aerosol concentrations and levels of viable micro-organisms were measured and compared to levels of the constituent endotoxin. Correlations between endotoxin levels and bacterial counts were higher for respirable endotoxin than for inhalable endotoxin. This can probably be explained by the sampling characteristics of the N-6 modification of the Andersen sampler, which measures predominantly micro-organisms of the respirable size fraction.[19,20] The fairly good correlation can be explained by constant bacterial growth and hence endotoxin lysis in waste water, which provides a simultaneous exposure close to sources. Walters *et al.*[21] found a correlation coefficient of 0.8 between countable bacteria and endotoxin in recycled wash water of a fibreglass insulation-manufacturing facility, which is comparable to findings in our study.

Endotoxin content of the sampled dust was equal for the inhalable and respirable size fractions. This deviates from results of the animal food industry[16] where 50% lower contents were found in respirable dust as compared with total, inhalable and thoracic dust ($D_{50}=8.5$ μm). Attwood *et al.*[22] found in samples from pig farms higher contents in thoracic dust, but similar levels in total and respirable dust. A lower mean endotoxin content in personally sampled dust in comparison with ambient air sampled dust in our study can be explained by the fact that personally sampled dust also consists of particles with lower endotoxin contents, derived from other sites (outside, technical department, control room, canteen).

In the fibre dehydration department of plant 2 the highest airborne endotoxin levels were found. Exposure appeared to be strongly related to process water temperature. Control measures at the source should include more thorough treatment of waste water before recycling, in combination with water cooling to inhibit bacterial growth. The open mills with textile sieves are known for spreading humid aerosols, and are therefore from an occupational hygiene perspective not recommendable.

Forster *et al.*[23] conducted a study in a sugar beet slicing plant and found high counts of micro-organisms and endotoxin levels up to 30 ng/m^3. In this industry also large amounts of water are recycled. Respiratory symptoms were reported and evaluated as work-related. Heederik *et al.*[24] found high levels of organic dust and fungi in a soft-paper plant, also with a large water recycling rate. Respiratory complaints were reported, and a decrease of pulmonary function over the week in exposed workers was found. These examples confirm our finding that recycling industrial process water forms an important source of exposure to micro-organisms and endotoxin, resulting in respiratory health complaints. Controlling this hazard will start with lowering the concentrations of micro-organisms (and endotoxin) in process water and reduction of exposure by replacing open mills by closed mills. A more detailed study is needed to investigate the impact of technical control measures on endotoxin exposure and workers' health.

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References

1. Hollander A, Heederik D, Kauffman H. Acute respiratory effects in the potato processing industry due to a bio-aerosol exposure. *Occup Environ Med* 1994; 51: 73–78.
2. Michel O, Duchateau J, Sergysels R. Effect of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. *J Appl Physiol* 1989; 66: 1059–1064.
3. Rylander R, Bake B, Fischer JJ, Helander IM. Pulmonary function and symptoms after inhalation of endotoxin. *Am Rev Respir Dis* 1989; 140: 981–986.
4. Ter Kuile WM. Vergleichsmessungen mit verschiedenen Geräten zur Bestimmung der Gesamtstaubkonzentration am Arbeitsplatz - Teil II. *Staub Reinhalt Luft* 1984; 44: 211–216. (In German, with a summary in English.)
5. Vrins E, Hofschreuder P. Sampling total suspended particulate matter. *J Aer Sci* 1983; 14: 318–322.

6. Douwes J, Versloot P, Hollander A, Heederik D, Doekes G. The influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol* 1995; 61: 1763 – 1769.
7. Hollander A, Heederik D, Versloot P, Douwes J. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. *Am Ind Hyg Assoc J* 1993; 54: 647 – 653.
8. Jones W, Morring K, Morey Ph, Sorenson W. Evaluation of the Andersen Viable Impactor for single stage sampling. *Am Ind Hyg Assoc J* 1985; 46: 294 – 298.
9. Smid T, Schokkin E, Boleij JSM, Heederik D. Enumeration of viable fungi in occupational environments: A comparison of samplers and media. *Am Ind Hyg Assoc J* 1989; 50: 235 – 239.
10. Andersen AA. New sampler for the collection, sizing, and enumeration of viable airborne particles. *J Bacteriol* 1958; 76: 471 – 484.
11. ACS committee on environmental improvement. Guidelines for data acquisition and data quality evaluation in environmental chemistry. *Anal Chem* 1980; 52: 2242 – 2249.
12. Helsel DR. Less then obvious. Statistical treatment of data below the detection limit. *Environ Sci Technol* 1990; 24: 1766 – 1774.
13. Kromhout H, Symanski E, Rappaport SM. A comprehensive evaluation of within- and between-worker components of occupational exposure to chemical agents. *Ann Occup Hyg* 1993; 37: 253 – 270.
14. Rappaport SM. Assessment of long-term exposures to toxic substances in air. *Ann Occup Hyg* 1991; 35: 61 – 121.
15. Attwood P, Brouwer R, Ruigewaard P, Versloot P, de Wit R, Heederik D, Boleij JSM. A study of the relationship between airborne contaminants and environmental factors in Dutch swine confinement buildings. *Am Ind Hyg Assoc J* 1987; 48: 745 – 751.
16. Smid T, Heederik D, Mensink G, Houba R, Boleij JSM. Exposure to dust, endotoxin and fungi in the animal feed industry. *Am Ind Hyg Assoc J* 1992; 53: 362 – 368.
17. Boleij JSM. Buringh E, Heederik D, Kromhout H. Occupational Hygiene of Chemical and Biological Agents. Amsterdam: Elsevier Science B.V., 1995.
18. Dutkiewicz J. Bacteria, fungi and endotoxin as potential agents of occupational hazard in a potato processing plant. *Am J Ind Med* 1994; 25: 43 – 46.
19. May KR. Calibration of a modified Andersen bacterial aerosol sampler. *Appl Microbiol* 1964; 12: 37 – 43.
20. Franzen H, Fißan HJ, Urban U. Eichung eines Andersen-Stack-Samplers unter Verwendung des Berglund-Liu-Aerosolgenerators. *Staub Reinhalt Luft* 1978; 38: 436 – 439. (In German, with a summary in English.)
21. Walters M, Milton D, Larsson L, Ford T. Airborne environmental endotoxin: a cross-validation of sampling and analysis techniques. *Appl Environ Microbiol* 1994; 60: 996 – 1005.
22. Attwood P, Versloot P, Heederik D, de Wit R, Boleij JSM. Assessment of dust and endotoxin levels in the working environment of Dutch pig farmers: a preliminary study. *Ann Occup Hyg* 1986; 30: 201 – 208.
23. Forster HW, Crook B, Platts BW, Lacey J, Topping MD. Investigation of organic aerosols generated during sugar beet slicing. *Am Ind Hyg Assoc J* 1989; 50: 44 – 50.
24. Heederik D, Burdorf L, Boleij JSM, Willems H, van Bilsen J. Pulmonary function and intradermal tests in workers exposed to soft-paper dust. *Am J Ind Med* 1987; 11: 637 – 645.

3. The influence of different filter elution methods on the measurement of airborne potato antigens[1]

Abstract

The effect of different materials and methods for filter elution on potato antigen yield was studied using parallel airborne dust samples from a potato processing plant. Sonication plus vortexing showed on average a 13% higher yield than gentle shaking. Type of filter (glass fibre or Teflon®), elution buffer (phosphate or bicarbonate) and the addition of 0.05% Tween-20 to the elution buffer had no significant effect on antigen yield. Addition of 0.5% Tween-20 instead of 0.05% resulted in a mean increase of 11%. We conclude that potato antigens can be readily eluted from airborne dust sampled on filters, and that the type of filter, elution buffer and elution conditions have little effects on elution efficiency. Similar studies should be performed for other antigens of interest as well.

[1] Jan-Paul Zock, Albert Hollander, Gert Doekes, Dick Heederik. *American Industrial Hygiene Association Journal* 1996; 57: 567–570.

Introduction

Assessment of exposure to airborne allergens and antigens is of growing importance in occupational and environmental epidemiological studies. In these studies, minimizing the exposure measurement error is desired to reduce exposure misclassification.[1] An optimal elution of filters used for sampling of airborne antigens contributes to a reduction of total measurement error.

Gordon *et al.*[2] compared two filter types and three elution methods with regard to the elution efficiency of airborne rat urine allergens. No significant differences between filter types or elution methods were found. Addition of 0.5% v/v of the mild detergent Tween-20 to the elution buffer caused a large increase of allergen concentrations, particularly for samples with low concentrations. As a consequence, a 5000-fold range in apparent allergen concentrations in field samples was reduced to a 100-fold range. Jensen *et al.*[3] compared three elution techniques using a phosphate buffer with 0.1% Tween-20 for filters from a high-volume sampler in order to optimize the yield of timothy and birch allergens. Elution of pulverized filters showed the largest yield of allergens, compared to 'descending elution' and elution by recycling. A higher concentration of Tween-20 (0.5%) led to an additional twofold increase of allergen yield.

In this study elution of potato antigens[4] from air sampling filters was studied. Potato antigens were studied because of our occupational health survey in the potato processing industry, during which a high level of antigenic activity could be demonstrated. The IgG_4 inhibition immunoassay used in these studies showed a high reproducibility when filter extracts were repeatedly tested. In the present study, the influence of the preceding procedures for filter extraction on the antigen yield was investigated. Two different filter types, two agitation methods and two elution buffers were compared and the effect of Tween addition was studied.

Material and methods

Air sampling

Air sampling was carried out in the fibre dehydration department of a potato processing plant.[4] A series of 16 airborne dust samples was taken simultaneously using a parallel sampling device as initially described by Eduard *et al.*[5] and as modified by Douwes *et al.*[6] with PAS 6 inhalable dust sampling heads[7] at a flow rate of 2 L/min. The flow rate of each sampling head was measured before and after each session. The inhalable size fraction was probably underestimated because it can be expected that relatively large particles were preseparated by the sampling device. Eight Whatman GF/A glass fibre filters and eight Millipore FALP02500 Teflon® filters were randomly allocated to the 16 available sampling heads. The sampling time was eight hours, resulting in a total volume of about 1 m^3 for each sample. Sampling was continuously repeated until 8 measurements were carried out, resulting in 128 samples. After sampling, filters were stored at −20°C before elution procedures.

Filter elution

All 128 filters were eluted within two days using $2^3 = 8$ different combinations of methods. Apart from filter type (glass fibre or Teflon), the other three bivariate main factors were: (1) elution buffer (phosphate or bicarbonate); (2) presence or absence of Tween-20; and (3) agitation method (shaking or sonication plus vortexing).

As elution buffers, phosphate buffered saline (PBS pH = 7.4: 0.14 M NaCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4 and 2.7 mM KCl) and bicarbonate buffer (BCB pH = 7.2: 0.086 M NaCl and 0.033 M $NaHCO_3$) were used. Both buffers were used either with or without the addition of 0.05% v/v Tween-20 (Merck, Darmstadt, Germany). 2.5 mL of the elution buffer was added to the filters, and subsequently subjected to either shaking or sonication plus vortexing. Shaking was done using a Gerhardt type LS-20 rocking apparatus, level four, for one hour at 4°C. Sonication plus vortexing was done as described by Houba *et al.*[8] Filters were successively vortexed for 2 minutes using a Retsch TM01 vortexer at maximal level, sonicated for 2 minutes in a water/ice mixture, vortexed for 5 minutes, and sonicated for 2 minutes. Eluates were isolated by centrifugation for

15 minutes at 5000*g* at 10°C. Supernatants were stored in glass tubes at −20°C.

ELISA inhibition assay

Samples were analyzed in duplicate on two different days for specific antigen content using an ELISA inhibition assay as described by Hollander *et al.*[4] Extract from wastewater of the same fibre dehydration department was used as coating antigen; previous experiments showed that inhibitory capacities of wastewater extract and airborne dust extract were similar. As antibody source, pooled serum was used, obtained from 18 workers with a high level of specific IgG_4 antibodies against extracts of airborne dust from the plant. Samples were thawed, diluted 1:80 in assay buffer (PBS with 0.05% v/v Tween-20 and 0.4% w/v gelatin), and centrifuged for 5 minutes at 1640 g. Six dilutions of samples were analyzed, from 1:160 to 1:5120. As controls, 12 serial dilutions of wastewater extract, four blanks (assay buffer and no subsequent addition of serum), four 0% inhibition controls (assay buffer) and four 100% inhibition controls (wastewater extract in excess) were included in each microtitre plate. Antigen concentrations were calculated using linear regression analysis with e−logit transformation. Concentrations were expressed as Antigen Units (AU/m^3), defined as the calculated dilution resulting in 50% inhibition.

Comparison of two different concentrations of Tween-20

To evaluate the effect of a higher concentration of Tween-20, elution efficiency was studied using PBS with either 0.5% or 0.05% v/v Tween-20. Two 24-hour measurements were also performed, as described under the section of this chapter titled 'air sampling', with glass fibre filters and Teflon filters. All 32 filters were eluted by sonication plus vortexing (see above). Supernatants of filters eluted with a buffer containing 0.5% Tween-20 appeared to be too troubled and were therefore centrifuged again. For three filters, supernatants remained trouble after a second centrifugation step; these supernatants were cleared by filtration through a 0.45 μm Millex-HA cellulose mixed ester filter (Millipore, Holsheim, France). Preliminary results showed that antigen concentration was not noticeably influenced by previous filtration procedures.

Statistical analysis

Data were analyzed using SAS statistical software, version 6.[9] Reproducibility of the inhibition test was evaluated by computing the coefficient of variation for analytical errors (CV_a) of duplicate measurements. Arithmetic means of duplicates were used in statistical analyses. The experiment was set up following a complete factorial design studying four main effects. Analysis of variance was performed using the General Linear Models Procedure of SAS. The distribution of antigen concentrations appeared to be skewed to the right, therefore, analyses of variance were performed using log-transformed values. Plausible first-degree interactions were included in the first model, together with the four main determinants, controlling for sampling session number and sampling head number. Interactions with an explained variance smaller than 1% were excluded from the model. The final model was tested for homogeneity of variance and for the presence of outliers.

Results

Comparison of filter types and elution conditions

The coefficient of variation (CV) of antigen analysis was on average 7.8% (range 0.1 to 25%), and did not differ significantly between the sampling sessions (Student's *t*-test; $P>0.05$). Antigen concentrations ranged from 738 to 1839 AU/m^3 ($N=128$). Analysis of variance with the first model, including 10 first-degree interactions, explained 93% of total variance ($R^2=93\%$). Five interactions were excluded because the partial R^2 was smaller than 1%. The results of the final reduced model are shown in Table 3.1. Variances appeared to be homogenous, and no outliers were found in the described model.

Table 3.1: Analysis of variance of ln-transformed antigen concentration with filter type, elution buffer, Tween-addition and agitation method. Estimated ratios are given for the four main effects. $N = 128$ samples.

Source	df	SS	MS	F	R^2 (%)
Model	55	4.513	0.082	13.2†	91.0
Error	72	0.448	0.006		

	df	Partial R^2 (%)	Levels	Ratio (e^{α})
Main effects:				
Sampling session	7	66.2†	—	—
Sampling head	15	6.4†	—	—
Filter type	1	0.0	Glass fibre vs. Teflon	1.00
Elution buffer	1	0.2	PBS vs. BCB	1.02
Tween-addition	1	0.1	0.05% Tween vs. no Tween	1.01
Agitation	1	8.4†	Sonication + vortexing vs. Shaking	1.13†
Interactions:				
Sampling session × Filter type	7	2.2	—	—
Sampling session × Elut. buffer	7	2.4	—	—
Sampling session × Tween-add.	7	1.7	—	—
Sampling session × Agitation	7	2.2	—	—
Filter type × Agitation	1	1.5†	—	—

df Degrees of Freedom
SS Sum of Squares
MS Mean Square
† $P<0.01$
PBS Phosphate Buffered Saline
BCB Bicarbonate Buffer

Only one main effect was significant: sonication plus vortexing resulted in a 13% higher antigen yield than shaking ($P<0.01$; partial $R^2 = 8.4\%$). Interaction of agitation method and filter type was highly statistically significant, and explained 1.5% of the total variance. Sonication plus vortexing showed a 9% higher yield for glass fibre filters and a 19% higher yield for Teflon filters, compared with shaking. Interactions of sampling session number with each of the four main effects had been included in the model of Table 3.1; each of them showed a partial R^2 larger than 1% (up to 2.4%). These interactions could not be attributed to differences in antigen level between sampling sessions.

Comparison of two different concentrations of Tween-20

The CV_a of antigen analysis in this experiment was on average 10.1% (range 0.5 to 26%), and was significantly different (Student's *t*-test; $P<0.05$) for the two sampling sessions (13% versus 7.5%). The total CV (CV_t), calculated with 8 quadruplicates amounted 11.3%. The estimated CV for measurement errors (CV_m) is therefore 5.1%. Antigen concentrations ranged from 467 to 829 AU/m^3 ($N=32$), and did not differ significantly between the two sampling sessions. Controlling for sampling head (16 levels) in the analysis of variance was initially done, but showed a loss in significance due to too many degrees of freedom for the model. The final model ($R^2=57\%$) contained one interaction term between sampling sessions and Tween concentration. Estimated ratio of 0.5% versus 0.05% Tween-20 was 1.11 ($P<0.01$; partial $R^2=16\%$). The interaction term was highly statistically significant and explained 33% of the total variance. When the interaction term was removed, or when sampling head was introduced in the model, the estimated ratio for Tween-20 remained the same. Filter type had no significant effect ($P=0.5$).

Discussion

In this study different materials and methods for filter elution were compared with regard to potato antigen yield. A complete factorial design made it possible to perform an orthogonal analysis of variance. Four main effects leading to $2^4=16$ different combinations were compared using a total of 128 samples, resulting in sufficient degrees of freedom. The measurement CV was estimated at 5%, indicating that samples were indeed more or less identical. Type of filter, elution buffer, and addition of 0.05% Tween-20 had no significant effect on antigen yield. An additional experiment showed that use of a tenfold higher concentration of Tween-20 (0.5%) resulted in an 11% higher elution efficiency. This is a very small difference compared to results described earlier.[2,3] Gordon *et al.*[2] reported an enhancement of 5 to 500 times caused by 0.5% Tween-20, dependent on the antigen concentration. Jensen *et al.*[3] reported a twofold enhancement using 0.5% Tween-20 compared to 0.1%.

Small differences were found between different agitation techniques. Sonication plus vortexing showed a 13% higher yield than gentle shaking. The signifi-

cant interactions between sampling session number and all main effects in our study could not be attributed to different antigen levels between the sampling sessions but might be explained by qualitative differences.

Douwes *et al.*[6] studied the influence of different elution conditions on the measurement of airborne bacterial endotoxin in a similar way. For these lipopolysaccharides (LPS), a sevenfold higher yield was noted when 0.05% Tween-20 was added to the elution medium, and a twofold difference due to different filter types. This indicates that for the measurement of LPS, choice of materials and methods for filter elution is of large importance for reducing measurement error and for proper comparisons between research groups. It appeared from our study that for the measurement of potato antigens, choice of filter type, elution buffer, Tween-addition and agitation method do not result in large differences in concentrations. It seems probable that the studied antigens are eluted easily and almost completely by the used procedures. Experiments from our own laboratory showed that potato-derived proteins were responsible for the described antigenic activity. Potato proteins are known to be highly soluble in water.[10] It can be assumed that our findings do not hold for other (less soluble) antigenic proteins. Moreover, the effects of different elution conditions on yield of antigens with carbohydrate structures are unknown. It is difficult to state which filter type and elution conditions will result in the highest yield of an antigen not previously studied in the ways described here. It is, however, not yet a common practice to perform such studies.

We conclude that potato antigens can be readily eluted, and different materials and conditions do not have large effects on elution efficiency. Similar studies should be performed for other airborne antigens and allergens measured in occupational health surveys.

Acknowledgements

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References

1. Armstrong BG. The effects of measurement errors on relative risk regressions. *Am J Epidemiol* 1990; 132: 1176–1184.
2. Gordon S, Tee RD, Lowson D and AJ Newman Taylor. Comparison and optimization of filter elution methods for the measurement of airborne allergen. *Ann Occup Hyg* 1992; 36: 575–587.
3. Jensen J, Poulsen LK, Mygind K, Weeke ER and B Weeke. Immunochemical estimations of allergenic activities from outdoor aero–allergens, collected by a high-volume sampler. *Allergy* 1989; 44: 52–59.
4. Hollander A, Heederik D and J Pothuis. Quantification of antigenic aerosol levels in the potato starch producing industry. *Ann Occup Hyg* 1994; 38: 911–918.
5. Eduard W, Lacey J, Karlsson K, Palmgren U, Ström G and G Blomquist. Evaluation of methods for enumerating microorganisms in filter samples from highly contaminated occupational environments. *Am Ind Hyg Assoc J* 1990; 51: 427–436.
6. Douwes J, Versloot P, Hollander A, Heederik D and G Doekes. The influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol* 1995; 61: 1763–1769.
7. Ter Kuile WM. Vergleichsmessungen mit verschiedenen Geräten zur Bestimmung der Gesamtstaubkonzentration am Arbeitsplatz – Teil II. *Staub Reinhalt Luft* 1984; 44: 211–216. (In German, with a summary in English.)
8. Houba R, van Run P, Heederik D and G Doekes. Wheat allergen exposure assessment for epidemiologic studies in bakeries using personal dust sampling and inhibition ELISA. *Clin Exp Allergy* 1996; 26: 154–163.
9. SAS Institute Inc. SAS User's Guide, version 6. Fourth edition. Cary, North Carolina: SAS, 1989.
10. Gorinstein S, Yamagata S and D Hadziyev. Electrophoretic separation of proteins and their amino acid composition in raw and processed potatoes. *J Food Biochem* 1988; 12: 37–49.

4. Airborne dust antigen exposure and specific IgG response in the potato processing industry[1]

Abstract

Background: High prevalences of work-related respiratory symptoms in relation to organic dust exposure have been reported in the potato processing industry, but the responsible effect mechanisms are not known.
Objective: To study the possible role of a Type III allergy in etiology.
Methods: Specific immunoglobulin G (IgG) and IgG_4 subclass antibodies against occupational airborne antigens were determined in sera from 131 potato processing workers and 36 non-exposed controls. Personal exposure to airborne antigens was measured, and a preliminary biochemical characterization was carried out.
Results: Specific IgG was detectable in almost all sera, but levels were significantly ($P<0.01$) higher in potato processing workers compared to controls. Specific IgG_4 was detectable in half of the workers' sera, but in none of the control sera. The antigens involved appeared to be heat-labile potato proteins. Antibody levels increased during the processing campaign in most workers, and this increase was dependent on the level of antigen exposure. Both the difference in IgG titres between the occupationally exposed group and the non-exposed group, and the exposure-related increase in specific IgG titres seemed to be mainly due to specific antibodies of the IgG_4 subclass. Specific antibodies showed a non-significant tendency to lower levels in workers with work-related respiratory symptoms.
Conclusion: Occupational respiratory exposure in the potato processing industry leads to a strong humoral immune response, most pronounced for IgG_4 subclass antibodies. Type III allergy is, however, unlikely to play a predominant role in the etiology of respiratory effects.

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[1] Jan-Paul Zock, Gert Doekes, Dick Heederik, Mariëlle van Zuylen, Peter Wielaard. *Clinical and Experimental Allergy* 1996; 26: 542–548.

Introduction

Processing of agricultural products involves occupational exposure to several constituents of organic dust[1,2], a well-known risk factor for several respiratory diseases.[3] In the potato processing industry high levels of dust, micro-organisms, endotoxin and antigens can be encountered, accompanied by high prevalences of work-related respiratory symptoms and peak flow patterns suggestive of reversible airway obstruction.[4-6] High levels of specific IgG_4 antibodies to airborne dust antigens were observed, whereas specific IgE antibodies could not be demonstrated and other IgG subclass antibodies were not measured. It was suggested that the observed work-related symptoms might be caused by an exceptionally high occupational endotoxin exposure, with full-shift average levels up to 30,000 Endotoxin Units per m^3.[6] An alternative possibility, that symptoms might be due to a Type III allergic reaction, could however not be excluded.

The presence of specific IgG antibodies against occupational antigens has been described for several occupational environments with organic dust exposure[1,7-9], including a sugar beet refinery in which Forster *et al.*[1] showed specific IgG reacting with both sugar beet extract, and with airborne dust extract. In sawmills with high levels of mould exposure, Eduard *et al.*[10] found high titres of IgG reacting with mould antigens in the employed wood trimmers. In the latter study, the specific immune response was also related with the occurrence of respiratory symptoms.[11] To our knowledge, no attention has thus far been given to the role of certain IgG subclasses in these occupational studies.

This report focuses on specific IgG and IgG_4 responses and the relation with respiratory health in 131 workers of four potato processing plants during the so-called 'campaign' from August to March[6]. The results were obtained as part of a study in which many other exposure and effect parameters were investigated as well. Serologic responses were related to personal antigen exposure levels, and a preliminary biochemical characterization of the antigens was performed.

Material and methods

Subjects of this study were 131 male employees from four facilities of a large potato processing company. Blood samples were drawn just before or at the start of the processing campaign (August 1992), and during the campaign (i.e. 7 to 17 weeks later in the period October – December 1992). From 109 workers, blood samples were taken in both periods. All subjects completed a questionnaire on occupational history, smoking habits and (work-related) respiratory and systemic symptoms. Control sera were obtained from 10 bakery workers[12], 6 members of a University Department and 20 randomly chosen healthy blood bank donors (with no information on age, gender and occupation).

Measurement of specific antibodies

Specific IgG and IgG_4 were determined by Enzyme Immuno Assay (EIA), with a coating of pooled airborne dust extract from one of the four plants[13] with a protein content of 150 μg per mg lyophilized material, as determined with the Bicinchoninic Acid reagent (Pierce, Rockford, USA). Microwells were coated overnight at 4°C with the antigen at a protein concentration of 4.5 μg/mL. Sera were tested at 1/500 and 1/5000, both in duplicate, in PBS containing 0.05% v/v Tween-20 and 0.2% w/v gelatin (PBTG; pH 7.4). After incubation for one hour at 37°C, binding of specific IgG and IgG_4 was determined by incubation for one hour at 37°C with peroxidase-labelled monoclonal mouse anti-human IgG or anti-human IgG_4 (Central Laboratory of the Blood transfusion service, Amsterdam, The Netherlands), respectively, diluted 1:1000 in PBTG, and finally an incubation for 30 minutes at 20°C with 2 mg/mL O-phenylenediamine (Sigma, St. Louis, USA) in citrate/phosphate buffer, pH 5.5, containing 0.015% v/v hydrogen peroxide. Optical density (OD) was read at 405 nm. Control sera were tested in the same way at 100-fold and 500-fold dilutions.

Antibody concentrations were expressed as relative titres, calculated by interpolation of the logit-transformed OD-values on a calibration line obtained in the same microtitre plate with a pool of 18 strongly positive sera[13], which was arbitrarily given an antibody titre of 100. The absolute titre of the pool, defined as the dilution leading to an OD of 0.25, was about 1/5000. As the detection limit we used the titre interpolated from a logit value −3. For samples with a

logit value above +3, the analysis was repeated at 5,000-fold and 50,000-fold dilutions.

Characterization of antigens

An IgG_4 inhibition assay[13] was performed with extracts prepared from peeled raw potatoes as used in the potato processing company, and with potato allergen preparations provided by 'Laboratorium Diephuis' (ALK Benelux, Groningen, The Netherlands). The latter were obtained from washed unpeeled raw consumption potatoes.

Characterization of antigenic determinants was performed by pre-incubation of airborne dust extract at different temperatures (20, 37, 60, 80 and 100°C) for 1 hour, and by pre-incubation with trypsin, pronase or periodate as described by Doekes *et al.*[14]

Personal antigen exposure

Repeated personal full-shift measurements of inhalable dust were performed.[6] Filters were eluted as described by Houba *et al.*[15], and antigenic activity was measured in an IgG_4 inhibition assay.[13] Samples were tested at fivefold dilution in duplicate. Antigenic activity was expressed in Relative Antigen Units (RAU), with the pooled airborne dust extract used as the standard, and arbitrarily given a value of 100 RAU/mL.

Results

Levels of specific antibodies

In Figures 4.1 and 4.2 the titres are presented of specific IgG and IgG_4 against airborne dust antigens, in workers from the four plants (during campaign), in bakers and in other controls. Specific IgG was detectable in 95% and 100%, and IgG_4 in 50% and 0% of the workers' and control sera, respectively.

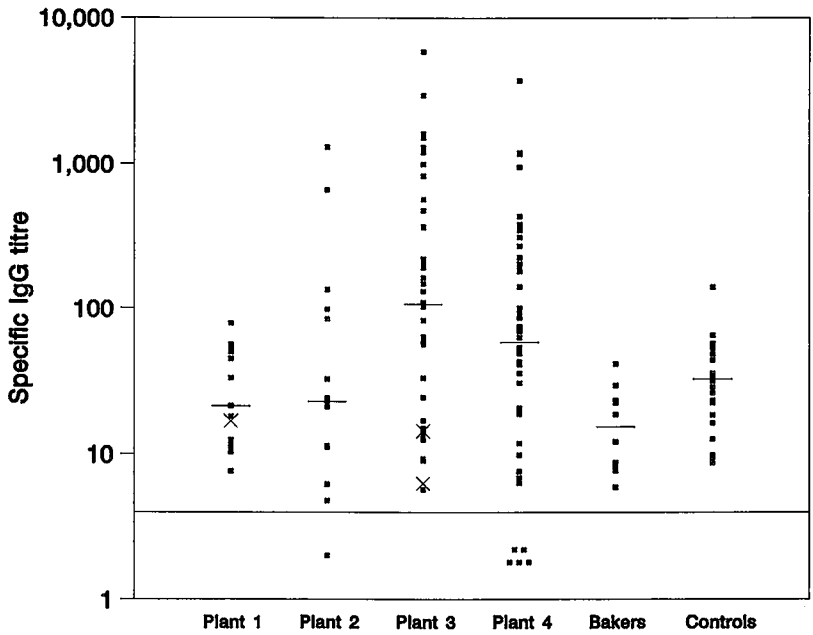

Fig. 4.1: Titres of specific IgG against airborne dust antigens in 117 sera from potato processing workers (during the potato processing campaign), 10 bakers and 26 other controls. Median levels are marked with '—'. Newly hired workers are marked with '×'. A titre of 4, indicated in the Figure, was used as the detection limit.

Antibody levels in potato workers' sera were higher than in control sera (Mann-Whitney *U*-test; $P<0.01$ for IgG and $P<0.001$ for IgG_4). Four new workers with no history of potato processing work showed low IgG levels not different from the controls, and no detectable IgG_4 levels. No association between appointment duration in the potato industry and antibody level was found. There was a strong association between specific IgG and IgG_4 levels for all 117 sera collected during the campaign (Spearman's correlation coefficient R being 0.80; $P<0.0001$). This association was weaker, but still highly significant, when the analysis was restricted to the 58 sera with detectable IgG_4 and IgG levels ($R=0.62$; $P<0.0001$). Figures 1a and 1b further show remarkably large differences in specific antibody levels between plants, being more pronounced for IgG_4.

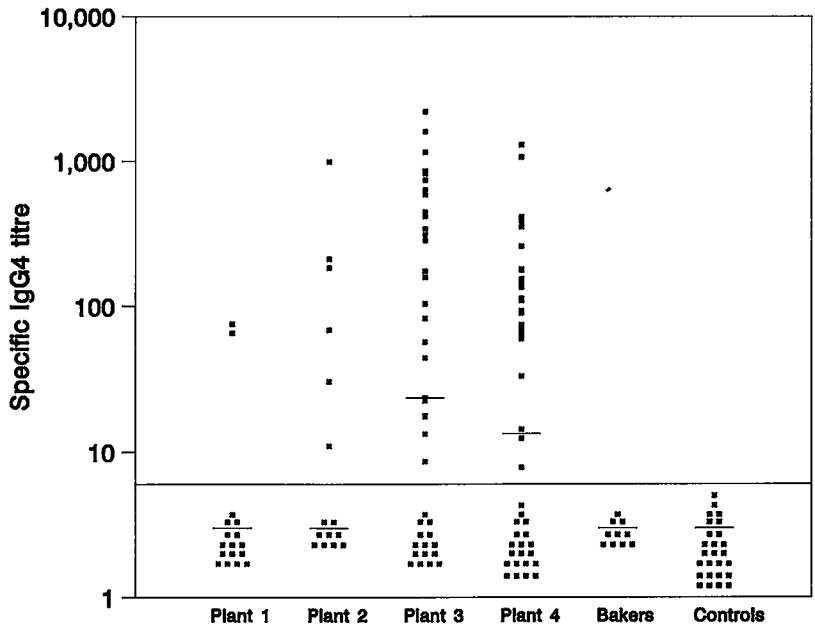

Fig. 4.2: Titres of specific IgG_4 against airborne dust antigens in 117 sera from potato processing workers (during the potato processing campaign), 10 bakers and 26 other controls. Median levels are marked with '—'. A titre of 6, indicated in the Figure, was used as the detection limit.

In the group of 109 workers from whom serum samples had been obtained both at the start and during the campaign, a small but significant increase during the campaign (on average 12%) was observed of both specific IgG (paired *t*-test; $P<0.01$) and specific IgG_4 ($P<0.005$) titre against airborne dust antigens. There was no relationship between time since the start of the campaign and change in IgG or IgG_4.

Characterization of antigens

Inhibition EIA's were performed to assess nature and biochemical characteristics of the antigens. A commercially available potato extract and an extract prepared in our own laboratory both inhibited IgG and IgG_4 reactions with the airborne dust extract completely, with dose-response inhibition curves that were parallel to those of the dust extract. This indicates that the large majority of the airborne dust antigens were potato components. The inhibitory capacities —defined as the protein concentration required for 50% inhibition— were similar for IgG_4, as shown in Fig. 4.3. For IgG, inhibitory capacities of the potato preparations were about 50% lower than of the dust extract (results not shown).

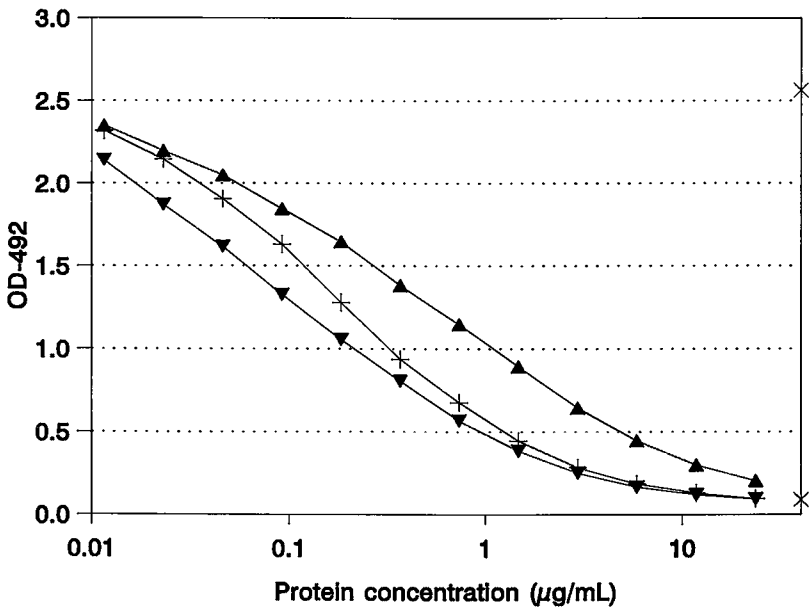

Fig. 4.3: Specificity of IgG_4 antibodies reacting with airborne dust extract from the potato processing industry. Inhibition EIA with a coating of airborne dust extract from the company studied, and IgG_4 antibodies in a pool of workers' sera. Inhibition curves are shown for a commercial potato extract (▲), a potato extract prepared in the laboratory (▼) and airborne dust extract itself (+). OD-values of 0% and 100% inhibition are shown with '×'.

IgG_4 inhibition EIA's were also performed with airborne dust extract after pre-incubation at different temperatures to study temperature sensitivity of the antigens; results are shown in Fig. 4.4. Antigenic activity was lower after pre-treatment at 60°C compared to 20° and 37°C. At 80° and 100°C most antigenic activity was lost. Pre-incubation of airborne dust extract with trypsin, pronase or periodate, had no significant effect on the antigenic activity in a subsequent IgG_4 inhibition EIA (results not shown).

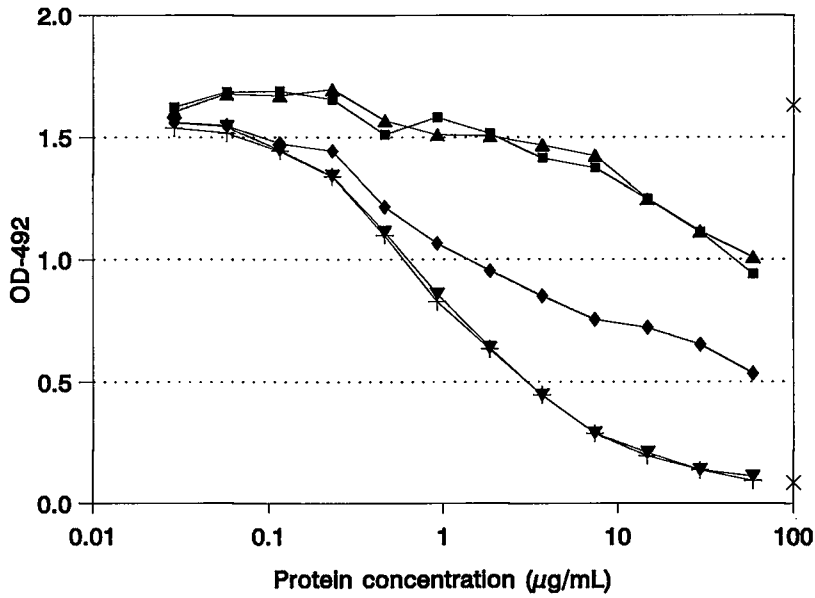

Fig. 4.4: Temperature sensitivity of IgG_4-binding antigens in airborne dust extract. IgG_4 inhibition EIA with a coating of airborne dust extract, and the same dust extract as inhibitor, after pre-incubation at 20° (▼), 37° (+), 60°(◆), 80° (■) and 100°C (▲). OD-values of 0% and 100% inhibition are shown with '×'.

Personal antigen exposure and immune response

Antigen measurements were performed in 186 personal dust samples from 123 workers, and showed a wide range in exposure levels. Large differences in exposure between the four plants were found, with the highest exposure levels in plants 2 and 3. In Fig. 4.5, a weak but significant ($P<0.05$) association between specific IgG level during the campaign and mean personal antigen exposure level can be seen (Spearman's $R=0.23$). For IgG_4, similar results were found ($R=0.29$; $P<0.005$).

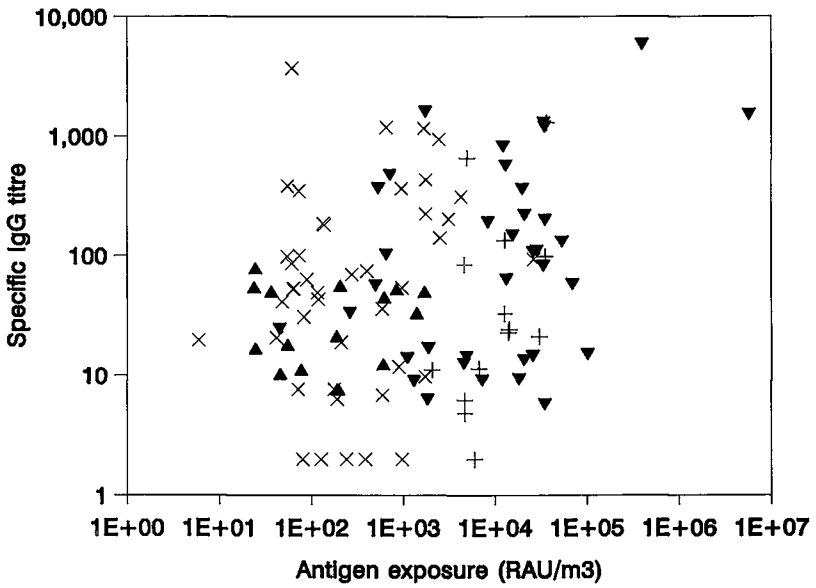

Fig. 4.5: Relation between antigen exposure and specific immune response: Scatterplot of specific IgG titre against airborne dust antigens, versus personal antigen exposure ($N=109$, Spearman's $R=0.23$).
▲ Plant 1. + Plant 2. ▼ Plant 3. × Plant 4.

A stronger association ($R=0.43$; $P<0.0001$) with exposure level was found for the relative change in IgG titre during the campaign. In Fig. 4.6 the relative change in IgG during the campaign is shown versus antigen exposure. For IgG_4, similar results were found ($R=0.45$; $P<0.0001$). When analyses were restricted to workers without detectable specific IgG_4 to airborne dust antigens, no significant relations between antigen exposure and specific IgG level were found. From Fig. 4.6, a threshold level for an increase of at least twofold in IgG of about 1000 RAU/m^3 might be suggested.

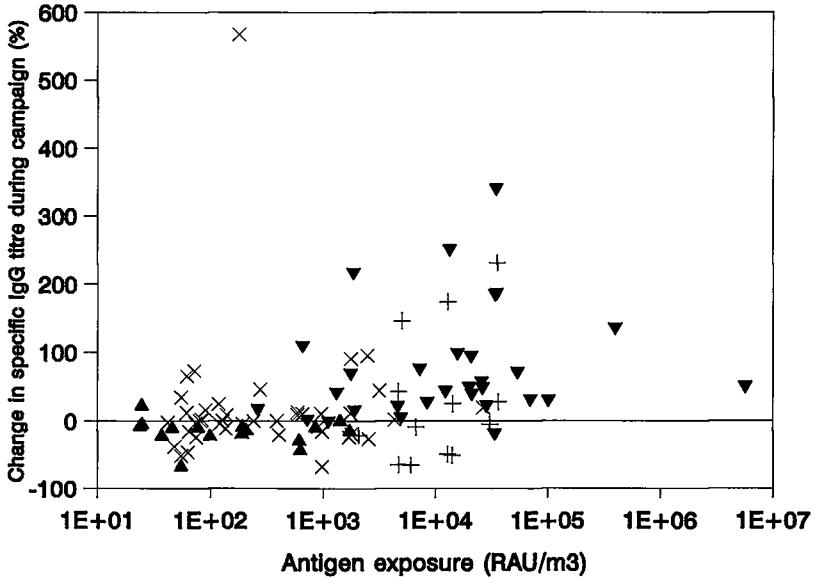

Fig. 4.6: Relation between antigen exposure and specific immune response: Scatterplot of increase of specific IgG titres against airborne dust antigens during the potato processing campaign, versus personal antigen exposure ($N=101$, Spearman's $R=0.43$). Titres below the detection limit were set at 2 before calculating the percentage change.
▲ Plant 1. + Plant 2. ▼ Plant 3. × Plant 4.

Workers were grouped according to their mean antigen exposure into three categories using 50- and 75 percentiles, corresponding with about 1000 and 10,000 RAU/m^3, arbitrarily as cut-off levels. In Table 4.1 percentages of subjects with an increase of specific IgG or IgG_4 titre are shown for the three exposure categories. Results are shown separately for smokers and non-smokers, because current smokers showed 3 to 4 times lower titres of specific IgG and IgG_4 compared to never- and former smokers.

Table 4.1: Percentages of workers with an increase of the specific IgG titre and IgG_4 titre during the processing campaign in three categories of antigen exposure using 50- and 75 percentiles as cut-off levels. Results are shown separately for smokers ($n=43$) and non-smokers ($n=58$).

Antigen exposure	SMOKERS			NON-SMOKERS		
	n	$\Delta(IgG)>0$	$\Delta(IgG_4)>0$	n	$\Delta(IgG)>0$	$\Delta(IgG_4)>0$
Low	24	25%	13%	26	46%	31%
Medium	10	50%	0%	15	73%	73%
High	9	67%	56%	17	94%	82%
χ^2 for trend		5.2*	5.3*		9.1†	11.9†

* $P<0.05$ † $P<0.01$

A dose-response relationship for both groups can be seen, being stronger for non-smokers, as indicated by the χ^2 values. Relative increase in IgG_4 titre was higher for non-smokers ($\chi^2=11.0$; $P<0.01$). For IgG, the relative increase was also higher ($\chi^2=2.6$), but not statistically significant. The Table further shows more frequent increases in specific IgG, compared to specific IgG_4.

In multivariate analyses, factors were highly correlated and it was not possible to identify which were the most important influences on IgG and IgG_4. Smoking appeared to be an effect modifier which meant that a stratified analysis had to be performed, but in each stratum the number of study objects was then too small to permit the examination of many factors at a time.

Immune response and work-related respiratory symptoms

Sixteen percent of the workers experienced at least one of the following work-related symptoms: cough (7%), phlegm (4%), shortness of breath (12%), wheezing (4%) and chest tightness (10%). These symptoms were reported by 18% of the current smokers and by 13% of the non-smokers. Titres of specific IgG in sera from workers with respiratory symptoms were lower than in sera from symptom-free workers, and 41% of the symptomatic workers had detectable specific IgG_4, compared to 54% of the symptom-free workers. Similar results were found for the specific work-related symptoms, but none of the differences was statistically significant ($P > 0.05$). No association was found between antigen exposure and respiratory symptoms, either. Thus, no positive association could be demonstrated between antigen exposure or specific IgG immune response and respiratory symptoms.

Discussion

In this study, a strong humoral immune response to airborne dust antigens was demonstrated among potato processing workers. Specific IgG was found in nearly all workers, and the IgG_4 subclass was found in about half of the workers. Control subjects with no history in potato processing work, and newly hired workers had low specific IgG levels and no detectable IgG_4. Characterization experiments revealed that the antigens involved were potato components, most probably heat-labile proteins. This indicates that work-related inhalatory exposure to potato dust may result in a specific IgG response to potato antigens, in which specific IgG_4 antibodies seem to predominate. The absence of periodate sensitivity indicates that probably no carbohydrate structures were responsible for antigenic activity. However, the antigen activity was also not affected by the proteolytic enzymes trypsin and pronase. For trypsin, this can be explained by the fact that potato tubers contain considerable amounts of trypsin-inhibitor[16], which strongly reduces the trypsin activity. For pronase, no direct explanation can be given, but it is suggested that potato tubers also contain other protease inhibiting proteins.[17,18]

Specific humoral immune responses to occupational antigens have been reported for other industries[1,8–10,19] as well. In this particular case it is noteworthy

that antibody levels in controls are negligible while in The Netherlands potato consumption is very common. The heat-lability of potato antigens, shown in Fig. 3, may explain this apparent discrepancy since potatoes are usually cooked before consumption. The heat-lability of the antigenic proteins has also been described for the allergens causing IgE mediated potato allergy.[20,21] Specific IgG_1 and IgG_4 antibodies against potato were nevertheless detectable in a significant number of sera from 213 unselected Dutch children, aged 3 months to 14 years [22], although specific IgG_4 levels to potato were in general low compared to other foods and compared to specific IgG_1. The used IgG_4 RAST however had a much lower detection limit compared to our study. For wheat flour antigens, specific IgG_4 antibodies can be found in individuals with an occupational airborne antigen exposure, namely bakery workers, but also in 15–30% of controls.[19] Thus wheat components might induce specific IgG_4 antibodies both after bread consumption and after wheat flour inhalation, whereas IgG_4 anti-potato antibodies only occur after inhalatory exposure.

A weak but significant relationship between antigen exposure and specific immune response was noted, and appeared to be mainly determined by specific IgG_4 responses. This corroborates our suggestion that the IgG_4 subclass accounts for the greater part of the specific IgG response. The relative change in specific antibody levels in the course of the campaign was stronger related to antigen exposure than the titre itself, probably because the specific antibody levels reflect the effect of several years of exposure. This assumption is supported by the absence of specific IgG_4 in newly hired employees after only a few months of exposure. Change in IgG by antigen exposure showed a dose response relationship between exposure and change in specific IgG. The correlation was stronger for non-smokers, suggesting that smoking is an effect modifier in this association. A multivariate analysis including smoking status as independent variable was therefore not appropriate. It is known from the literature that current smokers have lower serum IgG antibody levels[23,24], but it is notable that smoking also modifies the effect of antigen exposure on specific humoral immune response.

Although antigen exposure was similar in plants 1 and 4, specific $IgG_{(4)}$ titres were much higher in plant 4. The number of current smokers, 48% and 40% respectively, was not essentially different and cannot explain the observed differences. Endotoxin exposure levels however appeared to be on average three

times higher in plant 4 compared to plant 1[6], and this might be of importance for the observed differences in $IgG_{(4)}$ titres.

A positive relationship between specific IgG and work-related symptoms was not found, and Type III allergy is thus unlikely to play a predominant etiologic role. Moreover, IgG_4 appeared to predominate the specific immune response, and IgG_4 is neither able to react as a precipitating antibody in Type III allergy nor to activate the complement system.[25] The levels of $IgG_{(4)}$ in symptomatic workers tended to be somewhat lower in non-symptomatic workers; a protective effect of IgG_4 as 'blocking antibody'[25] can therefore not be excluded. This protective effect has also been observed among pig farmers.[26] Positive associations between specific IgG antibodies and respiratory symptoms, suggesting extrinsic alveolitis, have been observed in other settings[11,27], but the role of the IgG_4 subclass has not yet been elucidated.

We conclude that occupational respiratory exposure in the potato processing industry leads to a strong humoral immune response, which is mainly directed to potato antigens, probably heat-labile potato proteins, and is predominated by a specific IgG_4 response. This response, however, is probably of no relevance for the occurrence of respiratory symptoms. The presented data even suggest that specific IgG_4 production might protect against health effects.

Acknowledgements

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References

1. Forster HW, Crook B, Platts BW, Lacey J, Topping MD. Investigation of organic aerosols generated during sugar beet slicing. *Am Ind Hyg Assoc J* 1989; 50: 44–50.
2. Boleij JSM, Buringh E, Heederik D, Kromhout H. Occupational hygiene of chemical and biological agents. Amsterdam: Elsevier Science B.V., 1995.
3. Donham KJ, Thorne PS. Agents in organic dust: criteria for a causal relationship. *Am J Ind Med* 1994; 25: 33–39.
4. Hollander A, Heederik D, Kauffman H. Acute respiratory effects in the potato processing industry due to a bio-aerosol exposure. *Occup Environ Med* 1994; 51: 73–78.
5. Dutkiewicz J. Bacteria, fungi and endotoxin as potential agents of occupational hazard in a potato processing plant. *Am J Ind Med* 1994; 25: 43–46.
6. Zock JP, Heederik D, Kromhout H. Exposure to dust, endotoxin and micro-organisms in the potato processing industry. *Ann Occup Hyg* 1995; 39: 841–854.
7. Molina C. Occupational extrinsic allergic alveolitis. *Clin Immunol Allergy* 1984; 4: 173–192.
8. Virtanen T, Vilhunen P, Husman K, Mäntyjärvi R. Sensitization of dairy farmers to bovine antigens and effects of exposure on specific IgG and IgE titers. *Int Arch Allergy Appl Immunol* 1988; 87: 171–177.
9. Topping MD, Forster HW, Ide CW, Kennedy FM, Leach AM, Sorkin S. Respiratory allergy and specific immunoglobin E and immunoglobin G antibodies to reactive dyes used in the wool industry. *J Occup Med* 1989; 31: 857–862.
10. Eduard W, Sandven P, Levy F. Relationships between exposure to spores from *Rhizopus microsporus* and *Paecilomyces variotii* and serum IgG antibodies in wood trimmers. *Int Arch Allergy Immunol* 1992; 97: 274–282.
11. Eduard W, Sandven P, Levy F. Exposure and IgG antibodies to mould spores in wood trimmers: Exposure-response relationships with respiratory symptoms. *Appl Occup Environ Hyg* 1994; 9: 44–48.
12. Houba R, Heederik D, Doekes G, van Run P. Exposure-sensitization relationships for α-amylase in a baker's asthma study. *Am J Respir Crit Care Med* 1995; 151: A141.
13. Hollander A, Heederik D, Pothuis J. Quantification of antigenic aerosol levels in the potato starch producing industry. *Ann Occup Hyg* 1994; 38: 911–918.
14. Doekes G, Kaal MJH, van Ieperen-van Dijk AG. Allergens of *Pityrosporum ovale* and *Candida albicans*. II. Physicochemical characterization. *Allergy* 1993; 48: 401–408.
15. Houba R, van Run PEM, Heederik DJJ, Doekes G. Wheat allergen exposure assessment for epidemiologic studies in bakeries using personal dust sampling and inhibition ELISA. *Clin Exp Allergy* 1996; 26: 154–163.
16. Bryant J, Green TR, Gurusaddaiah T, Ryan CA. Proteinase inhibitor II from potatoes: Isolation and characterization of its protomer components. *Biochemistry* 1976; 15: 3418–3423.
17. Bracho GE, Whitaker JR. Characteristics of the inhibition of potato (*Solanum tuberosum*) invertase by an endogenous proteinaceous inhibitor in potatoes. *Plant Physiol* 1990; 92: 381–385.

18. Suh SG, Stiekema WJ, Hannapel DJ. Proteinase-inhibitor activity and wound-inducible gene expression of the 22-kDa potato-tuber proteins. *Planta* 1991; 184: 423 – 430.
19. Tiikkainen U, Klockars M. Clinical significance of IgG subclass antibodies to wheat flour antigens in bakers. *Allergy* 1990; 45: 497 – 504.
20. Nater JP, Zwartz JA. Atopic allergic reactions due to raw potato. *J Allergy* 1967; 40: 202 – 206.
21. Quirce S, Díez Gómez ML, Hinojosa M, Cuevas M, Ureña V, Rivas MF, Puyana J, Cuesta J, Losada E. Housewives with raw potato-induced bronchial asthma. *Allergy* 1989; 44: 532 – 536.
22. Calkhoven PG, Aalbers M, Koshte VL, Griffioen RW, van Nierop JC, van der Heide D, Aalberse RC. Relationship between IgG_1 and IgG_4 antibodies to foods and the development of IgE antibodies to inhalant allergens. I. Establishment of a scoring system for the overall food responsiveness and its application to 213 unselected children. *Clin Exp Allergy* 1991; 21: 91 – 98.
23. Gerrard JW, Heiner DC, Mink J, Meyers A, Dosman JA. Immunoglobulin levels in smokers and non-smokers. *Ann Allergy* 1980; 44: 261 – 262.
24. Finnegan MJ, Little S, Gordon DJ, Austwick PKC, Tee RD, Nunn AJ, Newman Taylor AJ. The effect of smoking on the development of allergic disease and specific immunological responses in a factory workforce exposed to humidifier contaminants. *Br J Ind Med* 1991; 48: 30 – 33.
25. Van der Zee JS, Aalberse RC. The role of IgG. In: Lessof MH, Lee TH, Kemeny DM, eds. Allergy: An international textbook. Chichester: John Wiley & Sons, 1987: 49 – 67.
26. Preller L. Respiratory health effects in pig farmers: Assessment of exposure and epidemiological studies of risk factors. Thesis. Wageningen Agricultural University, Wageningen, 1995: 109 – 116.
27. Nielsen J, Welinder H, Schütz A, Skerfving S. Specific serum antibodies against phtalic anhydride in occupationally exposed subjects. *J Allergy Clin Immunol* 1988; 82: 126 – 133.

5. Acute lung function changes and low endotoxin exposures in the potato processing industry[1]

Abstract

Work-related respiratory symptoms, acute lung function changes and personal endotoxin exposure were studied in 61 workers from a potato processing plant. According to their job title mean endotoxin exposure level, workers were divided into low (AM = 21 EU/m^3) and high (AM = 56 EU/m^3) exposure categories. Shortness of breath and chest tightness during work were reported by 18% and 16% of the workers, respectively, mainly in the low endotoxin exposure category. A total of 148 across-shift lung function changes were measured during three consecutive Afternoon shifts. The mean FEV_1 and MMEF showed a decrease over the work shift, being largest on the first working day after a 3-day absence from work. Workers exposed to high endotoxin levels showed a larger across-shift decrease in lung function than workers exposed to low endotoxin exposures, the effect being most pronounced on the first day after a 3-day absence from work. At the start of the second work shift, FVC, FEV_1 and MMEF were lower than at the start of the first work shift. This difference was larger for high exposed workers. High exposed workers with work-related respiratory symptoms showed an 8 – 10% across-shift change in FVC, FEV_1 and MMEF. We conclude that significant across-shift decreases in lung function of potato processing workers is related to endotoxin exposure levels above 53 EU/m^3 over 8 hr.

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[1] Jan-Paul Zock, Albert Hollander, Dick Heederik, Jeroen Douwes. *American Journal of Industrial Medicine* 1998; 33: 384 – 391.

Introduction

In the potato processing, industry high exposure levels of airborne dust containing bacteria, endotoxin and occupational antigens have been observed.[1-4] In the same studies prevalence rates of 16–46% for respiratory symptoms have been reported, among which shortness of breath and chest tightness predominated. Peak flow patterns suggestive of reversible airway obstruction have also been shown.[2] Flu-like symptoms such as malaise and muscular pains, even leading to sick-leave, have been reported in departments with an exceptionally high microbial exposure. The etiology of these respiratory effects has not yet been elucidated. An immediate Type I allergic response is improbable because specific IgE antibodies to occupational antigens have not been demonstrated in workers' sera.[2,4] Although a specific IgG response to occupational antigens has been observed, a Type III allergic reaction is not likely because this response was predominantly due to IgG_4 antibodies. It has even been suggested that IgG_4 as 'blocking antibody' may be protective against respiratory health effects.[5]

It has been suggested that exposure to endotoxin in this industry may play an important role in the etiology of these findings since clearly elevated endotoxin levels have been demonstrated. Associations between occupational endotoxin exposure and acute respiratory effects have been reported in a range of occupational settings.[6-8] In many industries, however, the endotoxin level is not well defined. Recently, Milton *et al.*[9] showed an exposure-response relationship between low endotoxin exposure ranging from 4 to 15 ng/m^3 and acute lung function changes in fiberglass manufacturing workers. It is still unclear above which endotoxin exposure level respiratory effects can be expected.

To investigate the etiological role of endotoxin in this industry in more detail, acute respiratory effects were studied in a potato processing plant with relatively low endotoxin exposure levels, as compared to three other plants.[3] This paper describes work-related respiratory symptom prevalences and across-shift lung function changes, and their relationships with endotoxin exposure in a potato processing plant.

Materials and methods

Population and questionnaire

The study population consisted of 61 male shift workers from a potato processing plant. A description of the production process can be found elsewhere.[3] Fifty-seven workers (93%) completed a self-administered questionnaire on occupational history in potato processing, smoking habits and the Dutch version of a questionnaire on respiratory symptoms of the British Medical Research Council[10], supplemented with questions on work-related respiratory symptoms. Symptoms were considered to be work-related if they occurred during work 'more frequently than normal'.

Lung function

Lung function measurements were performed in two periods. During the first period from 6 to 14 August 1990, which was before the start of the processing season, each worker was measured once to obtain a baseline lung function measurement. The second period, from 15 to 31 October 1990, was during the processing season. After a 3-day absence from work, each worker had lung function tests before and after work on three consecutive Afternoon shifts (3 p.m. – 11 p.m.). During this time of the day, lung function is in a relatively stable phase of the circadian rhythm.[11,12] Three p.m. lung function is at the top of the circadian rhythm, thus a decrease can be expected during the Afternoon shift.[13,14] Forced expiratory lung function measurements were performed using Vicatest-5 dry rolling-seal spirometers (Jaeger, Breda, The Netherlands), calibrated before and after measurement sessions. The measurements and procedures including adjustments for Body Temperature and Pressure Saturated with water vapour (BTPS), and data selection were performed according to the recommendations of the European Respiratory Society (ERS).[15] The following variables were registered: Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 second (FEV_1), Maximal Mid-Expiratory Flow (MMEF) and Peak Expiratory Flow (PEF).

Endotoxin exposure

Concurrent to the repeated lung function measurements, personal full-shift inhalable dust samples were taken using Dupont P-2500 portable constant-flow sampling pumps at a flow rate of 2.0 L/min in combination with PAS 6[16] samplers and Whatman GF/A glass fiber filters with a diameter of 25 mm. Sixty-eight of the 149 available dust filters (46%; randomly chosen but at least one per measured worker) were eluted with 5 mL pyrogen-free water (NPBI, Emmer-Compascuum, The Netherlands) and analyzed using a kinetic modification of the Limulus Amoebocyte Lysate assay as described by Hollander *et al.*[17] The factor to convert the outcome of the test (in ng/mL) to Endotoxin Units (EU/mL), relative to the US endotoxin standard EC-5, was equal to 12.0 EU/ng.

Statistical analyses

Data were analyzed using SAS version 6.[18] Workers were grouped in low and high endotoxin exposure categories according to their job title mean endotoxin exposure. Optimal grouping was evaluated by computing the variance components between and within categories using a two-way nested analysis of variance.[19]

Absolute changes in lung function were evaluated by using Student's paired *t*-test. Relative changes were computed by dividing the absolute change by the initial value, and multiplying by 100%. Relationships between endotoxin exposure, smoking and lung function change were analyzed using linear regression analyses (PROC REG). Models were tested for homogeneity of the variance and for the presence of outliers. Partial regression plots, residual plots and plots of Cook's *D* influence statistic vs. independent variables were visually inspected.

Results

Endotoxin exposure

In Table 5.1, descriptive statistics are given for the 68 personal endotoxin measurements: 90 % of the measurements was below 56 EU/m^3 ($\approx$5 ng/m^3). The overall ratio of within- to between-worker variance in log-transformed exposure (λ) was 2.1. This shows that day-to-day variation in exposure was relatively large. Technicians (group 1) and workers dealing with unloading /wash-

ing/grinding (group 4) showed the highest exposure levels, and the largest variations in exposure (GSD). Workers from drying departments (group 6) showed an intermediate mean exposure, but because the variation was small (GSD = 1.6) and the range overlapped with the range of group 3, group 6 was also placed in the low exposure category.

Analysis of variance using the low and high exposure categories revealed an exceptionally small (between-worker) within-category variance (GSD = 1.07). In combination with the observation of a more than twofold difference between the high and low exposure level categories, this is indicative of little overlap, and thus a high 'contrast', between the low and high endotoxin exposure categories.

Table 5.1: Descriptive statistics of personal endotoxin concentrations (EU/m^3) for eight job titles and two pooled job categories of potato processing workers.

Group	*N*	*K*	AM	GM	GSD	Range
1. Technicians	8	7	60.0	36.4	2.4	16.1 – 284
2. All-round	6	5	16.1	15.6	1.3	10.9 – 19.6
3. Protein department	16	13	20.7	18.9	1.8	10.4 – 59.5
4. Unloading / washing / grinding	15	12	53.4	45.7	2.3	10.6 – 120
5. Extraction / refinery	8	6	20.3	19.5	1.4	11.4 – 31.8
6. Drying departments	7	5	35.4	32.8	1.6	16.6 – 55.3
7. Sludge treatment	1	1	22.2	22.2	—	—
8. Weighing / sampling	7	5	13.5	12.2	1.8	6.8 – 29.9
All	68	54	32.9	24.9	2.0	6.8 – 284
Low = 2+3+5+6+7+8	45	35	21.2	18.9	1.6	6.8 – 59.5
High = 1+4	23	19	55.7	42.3	2.1	10.6 – 284

N Number of measurements
K Number of workers
AM Arithmetic Mean
GM Geometric Mean
GSD Geometric Standard Deviation

Respiratory health characteristics

Respiratory health characteristics for workers in the two exposure categories are shown in Table 5.2. Fourteen of the 57 workers (25%) reported at least one of four work-related respiratory symptoms, predominated by obstructive symptoms (shortness of breath and chest tightness). These symptoms seemed to be more prevalent in the low endotoxin exposure category.

Table 5.2: Demographic and respiratory health characteristics of 57 potato processing workers

	ENDOTOXIN EXPOSURE CATEGORY	
	Low ($n=37$)	High ($n=20$)
Age (years)	41 ± 7 *	39 ± 10
Employment in potato processing (years)	14 ± 7	13 ± 9
Smokers	21 (57%)	11 (55%)
Ex-smokers	8 (22%)	5 (25%)
Chronic respiratory symptoms	10 (27%)	5 (25%)
Work-related symptoms:		
Cough	4 (11%)	0 (0%)
Phlegm	1 (3%)	1 (5%)
Shortness of breath	9 (24%)	1 (5%) †
Chest tightness	9 (24%)	0 (0%) ‡
At least one of these symptoms	12 (32%)	2 (10%)
Baseline lung function§		
FVC % reference‖	106 ± 16	99 ± 14 †
FEV_1 % reference	102 ± 17	95 ± 15
MMEF % reference	88 ± 24	87 ± 26
PEF % reference	102 ± 27	91 ± 28

* Arithmetic mean ± standard deviation

† $P<0.1$ ‡ $P<0.05$ (Fisher-Exact test or two sample t-test)

§ $n=54$; 35 in low and 19 in high endotoxin exposure category

‖ According to the ERS[15]

Baseline lung function variables, relative to age- and height-specific reference values[15], tended to be higher in the low endotoxin exposure category. No appreciable difference in age, number of years employed in potato processing, smoking habits or prevalence of chronic respiratory symptoms existed between the two exposure categories.

Across-shift lung function changes

A number of 148 sets of across-shift lung function change from 61 workers was available for statistical analyses. For 35 workers, a complete set for all 3 days was available. Non-response occurred because of absence due to sickness leave or days off, because of measurements that failed to comply with the ERS criteria, or because of technical problems.

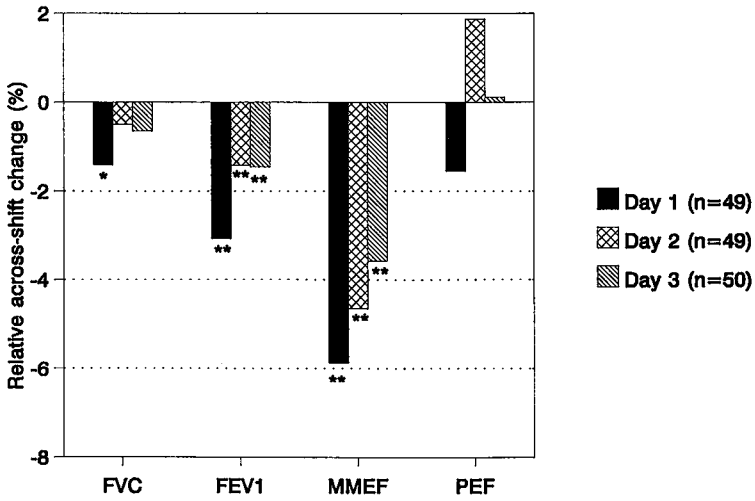

Fig. 5.1: Mean relative across-shift lung function change on three consecutive Afternoon shifts among 61 potato processing workers.
* $P<0.05$ ** $P<0.01$ (paired t-test)

Fig. 5.1 shows the mean relative across-shift lung function change on three consecutive Afternoon shifts. Mean FEV_1 and MMEF showed a significant decrease across each shift. Mean absolute decrease was equal to 0.06 – 0.12 L for the FEV_1 and 0.16 – 0.28 L/s for the MMEF. The largest decreases in function were observed on the first day. The PEF showed no consistent across-shift changes. Nevertheless, a mean decline in PEF was observed for the first day. Results for the 35 workers who performed measurements on all 3 days (105 changes) were essentially equal to the results presented in Fig. 5.1.

Pre-shift FEV_1 and MMEF on the second day were 1.9% and 4.8%, respectively, lower than pre-shift function on the first day, while the mean PEF was 5.6% higher. Furthermore, pre-shift PEF on the third day was 6.5% higher compared with the pre-shift PEF on day 2, while no significant differences in FEV_1 and MMEF were observed. No essential differences in pre-shift FVC between the 3 days were observed.

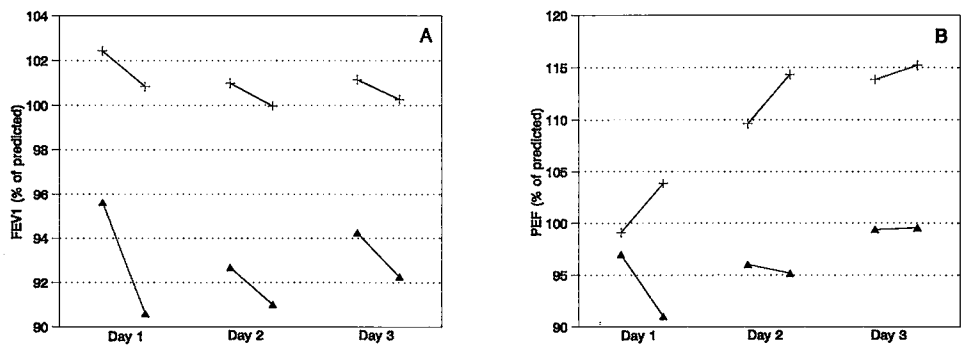

Fig. 5.2: Course in FEV_1 (A) and PEF (B) over three consecutive working days among 35 potato processing workers with a complete set of six lung function measurements. Results are shown averaged for 22 low exposed workers (indicated by ▲) and 13 high exposed workers (indicated by +).

Relationships between endotoxin exposure and lung function changes

In Fig. 5.2, the changes in FEV_1 and PEF over three working days are shown for the subset of 35 workers who provided a complete set on all 3 days. Lung function variables are expressed relative to age- and height-specific reference values.[15] Results are shown separately for 22 low and 13 high exposed workers. The graphs show pronounced differences in lung function changes between the two exposure categories. The PEF clearly increased over the study period in low exposed workers, while the PEF in high exposed workers showed no large changes. The across-shift decrease in FEV_1 appeared to be larger in high exposed workers, being most obviously for the first day. Fig. 5.2a further shows that 'baseline' FEV_1 was lower in the high endotoxin exposure category, which has also been noted in Table 5.2.

In Table 5.3, differences in relative across-shift lung function changes between the two exposure categories are presented. Difference in lung function change was clearly larger on the first day compared with days 2 and 3. In the high exposure category ($n=20$), mean decrease across the first shift was 2.3% for the FVC, 5.0% for the FEV_1, 8.6% for the MMEF and 8.0% for the PEF. The 24-hour change —defined as the difference between the pre-shift lung function values of the second and the first days— was also different between the endotoxin exposure categories. PEF in low exposed workers showed a mean increase of almost 10%, while the PEF in high exposed workers showed no appreciable change. Analyses using absolute across-shift changes showed similar results as presented for relative changes.

Table 5.3: Differences in relative across-shift lung function change (%) between high and low endotoxin exposure categories in 61 potato processing workers.

Variable	Day 1 ($n=49$)	Day 2 ($n=49$)	Day 3 ($n=50$)	24-hour change* ($n=51$)
ΔFVC	−1.5 *(−4.0;+1.0)*†	−1.0 *(−3.4;+1.3)*	−0.2 *(−2.6;+2.1)*	−0.5 *(−2.5;+1.6)*
ΔFEV_1	−3.2 *(−6.5;+0.2)*	−0.5 *(−3.0;+2.0)*	−0.7 *(−3.6;+2.2)*	−1.3 *(−3.3;+0.7)*
ΔMMEF	−4.5 *(−13;+3.6)*	+2.0 *(−3.0;+7.0)*	−2.0 *(−8.6;+4.6)*	−3.8 *(−10;+2.5)*
ΔPEF	−11 *(−20;−1.6)*	−1.2 *(−8.4;+6.1)*	−2.1 *(−8.0;+3.9)*	−9.9 *(−19;−1.0)*

* pre-shift value of the second day minus pre-shift value of the first day
† 95%-confidence interval

Table 5.4: Differences in relative across-shift lung function change (%) between high and low endotoxin exposure categories, stratified for current smoking and for presence of work-related respiratory symptoms in 61 potato processing workers.

		Current smoking		Work-related respiratory symptoms*	
	All ($n=61$)	yes ($n=32$)	no ($n=25$)	yes ($n=14$)	no ($n=43$)
ΔFVC	−0.7 *(−2.4;+1.1)*†	+0.5 *(−2.0;+3.0)*	−1.6 *(−4.4;+1.1)*	−6.0 *(−10;−1.9)*	−0.1 *(−2.1;+2.0)*
ΔFEV_1	−1.1 *(−3.3;+1.0)*	−0.1 *(−3.6;+3.5)*	−1.8 *(−4.5;+1.0)*	−5.0 *(−10;+0.4)*	−0.9 *(−3.4;+1.6)*
ΔMMEF	−0.7 *(−5.3;+3.9)*	−0.1 *(−7.3;+7.1)*	−2.4 *(−8.4;+3.7)*	+0.3 *(−12;+12)*	−2.6 *(−8.0;+2.9)*
ΔPEF	−4.0 *(−9.4;+1.3)*	−4.1 *(−12;+4.0)*	−5.2 *(−14;+3.2)*	+0.6 *(−13;+14)*	−6.7 *(−13;−0.2)*

* cough / phlegm / shortness of breath / chest tightness
† 95%-confidence interval

To study the influences of smoking and respiratory symptoms on the relationships between endotoxin exposure and lung function changes, lung function changes were averaged for each worker to avoid dependence in subsequent statistical analyses. In Table 5.4, differences in relative across-shift lung function change between high and low exposure categories are presented in four subsets of workers. Using data from all 61 workers, mean lung function change was more negative in the high exposure category. Data from four workers had to be deleted when stratifying for smoking habits or respiratory symptoms due to missing questionnaires. Differences in across-shift lung function change were larger in non-smokers. Among smokers, ΔFVC, ΔFEV_1 and ΔMMEF were similar for high and low exposed workers. Adjustment for current smoking in multiple regression models yielded differences between high and low exposure categories that were identical to unadjusted differences (results not shown).

Workers with work-related respiratory symptoms showed a threefold larger across-shift decrease in lung function compared to workers without these symptoms. Within the subset of 14 symptomatic workers, a large difference in ΔFVC and ΔFEV_1 between high and low exposure categories was observed. On the other hand, it was notable that effects on ΔMMEF and ΔPEF were only observed among workers without work-related symptoms. The percentage of smokers was equal in groups of workers with (57%) and without (56%) work-related respiratory symptoms. Workers with chronic respiratory symptoms also showed larger endotoxin-related effects on ΔFVC and ΔFEV_1, but much less pronounced than for work-related symptoms (results not shown).

Discussion

In this study, across-shift lung function changes were associated with the level of endotoxin exposure. Personal exposure measurements revealed relatively low endotoxin concentrations, confirming previously reported results from this particular plant.[3] On the basis of job title mean exposure levels, a high and a low exposure category could be set. Both categories could be considered as homogeneous exposure groups. There are, however, a number of items regarding our study that need to be addressed.

Proper comparisons between laboratories with regard to endotoxin exposure levels are often difficult to make. Sampling, elution, storage and analysis are all recognized factors that may influence endotoxin concentrations.[20,21] An experimental study using dust samples from a similar potato processing plant of the same company[21] showed a sevenfold higher endotoxin yield when 0.05% v/v of the detergent Tween-20 was added to the elution medium pyrogen-free water. This implies that when comparing our data to results from studies in which detergents were used in elution procedures, endotoxin exposure concentrations were most probably underestimated in our study. Furthermore, it cannot be excluded that other bacterial components like peptidoglycan[22,23] play a part in observed lung function changes since a previous study showed that endotoxin levels were related to bacterial counts.[3]

The increase in peak flow across the study period, being pronounced in workers with low endotoxin exposures, is probably due to a learning effect. This may have caused changes in the shape of the flow-volume curve.[24] As a result, a decrease in FEV_1 and MMEF is related to an increase in PEF. This 'effort dependence' has been observed in other studies using repeated spirometry, both in industrial workers[7] and in school children[25]. In our study, differences in ΔFVC, ΔFEV_1 and ΔMMEF between high and low exposure categories were most probably not overestimated due to this phenomenon.

FEV_1 and MMEF were significantly lower at the start of the work shift on the second day (16 hr after exposure), than at the start of the work shift on the first day. This can partly be explained by the effort dependence described before, since the PEF showed a considerable increase. However, this is not true for the high exposure category since the PEF showed no 24-hr change in this subset. Apparently, a complete recovery of the large lung function decrease gained during the first work shift was not achieved. This effect is consistent with a delayed inflammatory response to endotoxin, as described previously for patients with mild asthma.[26] Milton *et al.*[9] also showed endotoxin-related lung function decreases from pre-shift to arising next day, 16 – 20 hr after exposure.

Lung function decrease was roughly twofold larger across the first work shift after a 3-day absence from work, compared to the second and third shifts. The most likely explanation is that pre-shift lung function was lower on the second and third working day than on the first day, showing an enduring decrease. For this reason, lung function drops less during subsequent working days. Further-

more, this effect is consistent with findings that endotoxin-induced symptoms were most severe on Mondays and became milder the following days.[27] The occurrence of a short-term adaption or 'tolerance' has been reviewed previously[28], and might play a role in the explanation of this phenomenon.

Smoking appeared not to confound results since correction in a multiple regression analysis did not change crude differences between workers with high and low endotoxin exposures. There are some suggestions from the literature that smoking might modify the relationship between endotoxin exposure and lung acute function change. Haglind and Rylander[29] observed that 17 smoking cotton workers had a larger endotoxin-related decrease in FEV_1 than 11 non-smoking cotton workers. However, we observed that endotoxin-related decrease in lung function was larger in non-smokers, and these findings contradict the quoted study. Nevertheless, both studies are probably too small to draw conclusions regarding the interfering effect of smoking. There were indications for stronger reactions in symptomatic workers since endotoxin-related effects on FVC and FEV_1 seemed to be larger in workers with work-related respiratory symptoms. Michel *et al.*[30] reported that asthmatic subjects showed bronchial obstruction at lower endotoxin doses compared to normal subjects. From our results it can be suggested that work-related symptoms are more specific than chronic respiratory symptoms in the determination of endotoxin-susceptible workers. Interpretation should be done with some caution, because numbers were small and this pattern was not consistent for all four lung function variables.

In a comparable study in the animal feed industry[7] using the same protocol for lung function measurements and similar equipment, it was shown that across-shift decrease in MMEF was related to endotoxin exposure levels above 15 ng/m^3. Exposure-related effects on FVC, FEV_1 and PEF could not be detected. Since mean PEF increased during the work shift in high exposed workers, effort dependence might have played a role in the observed endotoxin-related decrease in MMEF. Across-shift lung function decrease in this study was also largest on the first day after an absence from work, but associations with endotoxin exposure using exclusively the first day were not described. Although elevated prevalence rates of work-related respiratory symptoms were reported, analyses using only symptomatic workers were not provided.

Our study clearly showed that endotoxin exposure related across-shift lung function changes exist. On the first day after an absence from work, mean lung function decrease in workers exposed to high endotoxin concentrations (53 – 60 EU/m^3) was equal to 5% for the FEV_1 and 8% for the PEF. In healthy subjects, lung function decline from 3 to 11 p.m. due to the circadian rhythm is 1 – 2% for the FEV_1[13] and about 3% for the PEF[14]. However, our design does not allow a statement on possible endotoxin-related effects in the low exposure category.

The exposure related effects suggest that endotoxin-related effects on across-shift lung function change can be expected above 53 EU/m^3 ($\approx$4.5 ng/m^3) over 8 hr. In a fiberglass manufacturing facility, Milton *et al.*[9] found peak flow changes $\geq$5% at endotoxin levels of 4 – 15 ng/m^3, which is in concordance with our study. The endotoxin source in the quoted study was recycled process water, which also appeared to be the major source in this industry.[3] A difference between these two industrial settings, however, is the absence of other organic dust components in the fiberglass plant. To our knowledge, 53 EU/m^3 in our study and 40 EU/m^3 in the fiberglass study[9] are the lowest reported concentrations at which a significant lung function decline was observed. Evident associations between endotoxin exposure levels below 25 EU/m^3 and acute lung function change have not been published. Sigsgaard *et al.*[31] reported an exposure-response trend for across-shift Monday FVC and FEV_1 in 67 Danish garbage workers at endotoxin levels of $<$1 to 2.5 ng/m^3. However, lung function change appeared to be more strongly related to dust than to endotoxin exposure. Sama *et al.*[32] found no effects at a geometric mean endotoxin exposure of 9 EU/m^3 for FEV_1 change among automotive workers.

We conclude that across-shift lung function decrease in potato processing workers is related to endotoxin exposure levels above 53 EU/m^3. Endotoxin-related effects seem to be larger in non-smokers and larger on the first day after a period absence from work.

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References

1. Dutkiewicz J. Bacteria, fungi and endotoxin as potential agents of occupational hazard in a potato processing plant. *Am J Ind Med* 1994; 25: 43–46.
2. Hollander A, Heederik D, Kauffman H. Acute respiratory effects in the potato processing industry due to a bio-aerosol exposure. *Occup Environ Med* 1994; 51: 73–78.
3. Zock JP, Heederik D, Kromhout H. Exposure to dust, endotoxin and micro-organisms in the potato processing industry. *Ann Occup Hyg* 1995; 39: 841–854.
4. Zock JP, Doekes G, Heederik D, Van Zuylen M, Wielaard P. Airborne dust antigen exposure and specific IgG response in the potato processing industry. *Clin Exp Allergy* 1996; 26: 542–548.
5. Van der Zee JS, Aalberse RC. The role of IgG. In: Lessof MH, Lee TH, Kemeny DM (eds): Allergy: An international textbook. Chichester: John Wiley & Sons, 1987: 49–67.
6. Donham K, Haglind P, Peterson Y, Rylander R, Belin L. Environmental and health studies of farm workers in Swedish confinement buildings. *Br J Ind Med* 1989; 46: 31–37.
7. Smid T, Heederik D, Houba R, Quanjer PhH. Dust- and endotoxin related acute lung function changes and work-related symptoms in workers in the animal feed industry. *Am J Ind Med* 1994; 25: 877–888.
8. Milton DK, Amsel J, Reed CE, Enright PL, Brown LR, Aughenbaugh GL, Morey PhR. Cross-sectional follow-up of a flu-like respiratory illness among fiberglass manufacturing employees: Endotoxin exposure associated with two distinct sequelae. *Am J Ind Med* 1995; 28: 469–488.
9. Milton DK, Wypij D, Kriebel D, Walters MD, Hammond K, Evans JS. Endotoxin exposure-response in a fiberglass manufacturing facility. *Am J Ind Med* 1996; 29: 3–13.
10. Biersteker K, Van Dijk WH, Eissens JBMF, Van Geuns HA. Ervaringen met geneeskundig onderzoek op CARA bij gemeentepersoneel te Rotterdam in 1970–1971. *T Soc Geneesk* 1974; 52: 158–162. (In Dutch, with a summary in English.)
11. Walford J, Lammers B, Schilling RSF, Van den Hoven van Genderen D, Van der Veen YG. Diurnal variation in ventilatory capacity. An epidemiological study of cotton and other factory workers employed on shift work. *Br J Ind Med* 1966; 23: 142–148.
12. Lewinsohn HC, Capel LH, Smart J. Changes in forced expiratory volumes throughout the day. *Br Med J* 1960; 45: 462–464.

13. Love RG. Lung function studies before and after a work shift. *Br J Ind Med* 1983; 40: 153–159.
14. Cinkotai FF, Sharpe TC, Gibbs ACC. Circadian rhythms in peak expiratory flow rate in workers exposed to cotton dust. *Thorax* 1984; 39: 759–765.
15. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernoult JC. Lung volumes and forced ventilatory flows. *Eur Respir J* 1993; 6, Suppl. 16: 5–40.
16. Ter Kuile WM. Vergleichsmessungen mit verschiedenen Geräten zur Bestimmung der Gesamtstaubkonzentration am Arbeitsplatz – Teil II. *Staub Reinhalt Luft* 1984; 44: 211–216. (In German, with a summary in English.)
17. Hollander A, Heederik D, Versloot P, Douwes J. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. *Am Ind Hyg Ass J* 1993; 54: 647–653.
18. SAS Institute Inc. SAS User's Guide, version 6. Cary, North Carolina: SAS, 1989.
19. Kromhout H, Tielemans E, Preller L, Heederik D. Estimates of indiviudal dose from current measurements of exposure. *Occup Hyg* 1996; 3: 23–39.
20. Walters M, Milton D, Larsson L, Ford T. Airborne environmental endotoxin: a cross-validation of sampling and analysis techniques. *Appl Environ Microbiol* 1994; 60: 996–1005.
21. Douwes J, Versloot P, Hollander A, Heederik D, Doekes G. The influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol* 1995; 61: 1763–1769.
22. Verhoef J, Kalter E. Endotoxic effects of peptidoglycan. In: Ten Cate JW, Büller HR, Sturk A (eds). Bacterial endotoxins: Structure, biomedical significance, and detection with the Limulus Amebocyte Lysate test. New York: Alan R. Liss, 1985: 101–112.
23. Burroughs M, Rozdzinski E, Geelen S, Tuomanen E. A structure-activity relationship for induction of meningeal inflammation by muramyl peptides. *J Clin Invest* 1993; 92: 297–302.
24. Krowka MJ, Enright PL, Rodarte JR, Hyatt RE. Effect of effort on measurement of forces expiratory volume in one second. *Am Rev Respir Dis* 1987; 136: 829–833.
25. Hoek G, Brunekreef B. Time trends in repeated spirometry in children. *Eur Respir J* 1992; 5: 553–559.
26. Michel O, Ginanni R, Le Bon B, Content J, Duchateau J, Sergysels R. Inflammatory response to acute inhalation of endotoxin in asthmatic patients. *Am Rev Respir Dis* 1992; 146: 352–357.
27. Rylander R, Imbus HR, Suh MW. Bacterial contamination of cotton as an indicator of respiratory effects among card room workers. *Br J Ind Med* 1979; 36: 299–304.
28. Ziegler-Heitbrock HWL, Blumenstein M, Käfferlein E, Kieper D, Petersmann I, Endres S, Flegel WA, Northoff H, Riethmüller G, Haas JG. *In vitro* desensitization to lipopolysaccharide suppresses tumor necrosis factor, interleukin-1 and interleukin-6 gene expression in a similar fashion. *Immunology* 1992; 75: 264–268.
29. Haglind P, Rylander R. Exposure to cotton dust in an experimental cardroom. *Br J Ind Med* 1984; 41: 340–345.
30. Michel O, Duchateau J, Sergysels R. Effect of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. *J Appl Physiol* 1989; 66: 1059–1064.
31. Sigsgaard T, Malmros P, Nersting L, Petersen C. Respiratory disorders and atopy in Danish refuse workers. *Am J Respir Crit Care Med* 1994; 149: 1407–1412.

32. Sama SR, Kriebel D, Woskie SR, Eisen EA, Virji MA, Hammond SK, Christiani DC, Milton DK. Machining fluid exposure and short-term respiratory responses among automotive workers. Tenth International Symposium on Epidemiology in Occupational Health Como, Italy 1994: 184. (Abstract.)

6. The influence of type of work shift and host factors on the relationship between occupational endotoxin exposure and acute peak flow changes[1]

Abstract

There is limited knowledge which internal and external factors influence respiratory effects of occupational endotoxin exposure. Relationships between endotoxin exposure, work-related respiratory symptoms and acute peak flow changes were studied in 97 shift workers in the potato processing industry. For each worker, across-shift peak flow changes were determined for Morning, Afternoon and Night shifts. A higher endotoxin exposure was associated with an increased prevalence of work-related symptoms, a smaller PEF increase across the Morning shift and a larger PEF decrease across Afternoon and Night shifts. The largest effects occurred during the Afternoon shift. Consistent differences between smokers and non-smokers, and between atopics and non-atopics were not observed. We conclude that endotoxin exposure in the potato processing industry is related to across-shift peak flow changes and the occurrence of work-related respiratory symptoms. The host factors smoking and atopy are no important modifying factors in these relationships. In respiratory health studies among shift workers, it is important to investigate all work shifts before drawing definitive conclusions about exposure-response relationships.

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Introduction

Acute respiratory effects of airborne endotoxin exposure are well recognized. Endotoxin-related lung function decrease has been shown in several challenge studies.[1,2] Moreover, in a number of occupational settings associations between endotoxin exposure levels and lung function change across the working day have been observed.[3–5] However, the influence of host factors such as smoking, atopy and respiratory health status on these relationships has not yet been elucidated. In a few experimental studies the role of host factors has been addressed. Blaski *et al.*[6] conducted a challenge study among 20 healthy non-smokers who inhaled corn dust extract with 30 μg of endotoxin. Atopy was defined as a skin test response to ≥ 2 environmental allergens. Significant differences in acute airway responses between atopics and non-atopics were not observed. In an other controlled study, Haglind and Rylander[7] observed a larger endotoxin-related decrease in FEV_1 among 17 smoking cotton workers compared with 11 non-smoking cotton workers. Michel[8] found in a lipopolysaccharide challenge study that lung function decrease in 38 asthmatics was larger than in 13 'normal subjects'.

In addition to host factors, also little is known about endotoxin-related respiratory effects during different types of work shifts. Airway calibre and as a result lung function is known to be subject to a circadian rhythm.[9–11] Workers on other than day shifts will normally experience a phase shift of this circadian cycle to correspond with their own sleep-wake cycle. Several days are necessary to make this adjustment, and some individuals adapt more completely than others.[12] Many studies have been conducted to assess lung function changes across the working day[3–5,11,13–15], but few considered different types of work shifts. However, already in 1966 the most comprehensive study on across-shift changes in $FEV_{0.75}$ among 473 shift workers employed in three cotton mills was reported.[16] Different magnitudes of lung function change for Early, Late and Night shift were observed, being dependent on job title and presence of byssinosis. Later but smaller studies[14,17,18] confirmed the observation of different magnitudes of across-shift change for different work shifts, also for other lung function indices.

To our knowledge, only one study addressed acute lung function changes in relation to occupational endotoxin exposure for different types of work shifts.

Milton *et al.*[5] studied peak flow changes across Morning and Night shifts in a fiberglass manufacturing facility. During the Night shift a larger number of workers showed a PEF decrease ≥5% compared to the Morning shift. During both shifts, an endotoxin-related exposure-response relationship with a PEF decrease ≥5% seemed present. Potentially modifying effects of type of work shift on the exposure-response relationship were not clearly evaluated, but it could be deduced from the data that the slope of these exposure-response relationships was similar for the Morning and Night shift.

As part of a larger study on respiratory health in potato processing workers, repeated peak flow measurements were performed among 97 workers. In this industry, a large range in endotoxin exposure across different plants and different departments has been observed[19], and endotoxin-related lung function changes across the Afternoon shift have been described[20]. This report focuses on across-shift peak flow changes and their relationships with endotoxin exposure in four potato processing plants. Special attention is given to the influence of type of work shift on these relationships. To identify workers who may be susceptible to endotoxin, the role of the host factors smoking and atopy was also investigated.

Subjects and methods

Population and questionnaire

The study population comprised 97 male shift workers from four plants of a large potato processing company of whom complete peak flow records were available.[21] The plants were in continuous operation from August to March. Three work shifts were distinguished: Morning (7 a.m. – 3 p.m.), Afternoon (3 – 11 p.m.) and Night (11 p.m. – 7 a.m.). In one of the four plants shifts changed one hour earlier (6 a.m., 2 p.m., 10 p.m.). The sequence of shift rotation was Afternoon → Morning → Night → Afternoon, etc. A work period lasted alternately three or four days, with a subsequent leisure period of two days (after Morning and Afternoon shift) or three days (after Night shift).

Workers completed a self-administered questionnaire with items on occupational history, smoking habits, and the Dutch version of a questionnaire on respiratory symptoms of the British Medical Research Council.[22] Questions on

work-related respiratory symptoms, determined as 'occurring during work more frequently than normal' were also included.

Peak flow measurements

Peak Expiratory Flow (PEF) was measured using mini-Wright peak flow meters three times (leisure days) or four times (work days) daily for a 23-day period in August/September 1992. On work days target times were (1) just after rising, before work; (2) in the middle of the work shift; (3) directly after the work shift and (4) before going to sleep. On leisure days target times were (1) just after rising; (2) in the middle of the day and (3) before going to sleep. Exact time of measurement was also recorded. On each occasion, three manoeuvres were performed[23,24] and recorded in the diary. The Coefficient of Variation (CV) between the three manoeuvres was calculated for each occasion and averaged per subject to evaluate measurement precision. Further analyses were performed using the highest of the three manoeuvres. Peak flow-time graphs were made for each worker, and visually inspected to detect obvious data errors. To assess PEF variability within days, Amp/Mean[12,25-28] was calculated for the different work shifts and leisure days.

Peak flow measurements closest to the start and to the end of the work shift were used to calculate across-shift changes in peak flow. Deviations of prescribed measurement times from start or end of the shift had to be within two hours. Peak flow change on leisure days was calculated using times similar to those of the afternoon shift (3 – 11 or 2 – 10 p.m.).

Atopic status

Serum samples were available from 93 workers; 85 workers were sampled both before and during the processing campaign. Total IgE as well as specific IgE to five common aero-allergens (house dust mite, grass pollen, birch pollen, cat dander and dog dander) were determined in all 178 sera as described by Doekes *et al.*[29] Workers were considered atopic if elevated specific IgE to at least one allergen was detected, either before or during the campaign. A second definition of atopy required a total IgE concentration $\geq$100 Units/mL, either before or during the campaign.

Endotoxin exposure

Results of endotoxin exposure measurements have been described elsewhere.[19] Briefly, 195 personal full-shift inhalable endotoxin exposure measurements were performed among 123 workers. Large differences in exposure level were observed between plants and between job categories. A job exposure matrix (JEM) based on a categorization of job by plant with 27 categories was developed. The arithmetic mean endotoxin exposure for each category was used as exposure estimate for workers in each group. Thus, for each of the 97 workers in this study endotoxin exposure was assessed on the basis of his plant and job category, and ranged from 53 to 8,167 Endotoxin Units per m^3 (EU/m^3). The overall Geometric Mean exposure of the 97 workers as estimated using the JEM was equal to 534 EU/m^3 (Geometric Standard Deviation 3.24). Because the distribution of endotoxin exposure was skewed to the right, all analyses were performed using ln-transformed concentrations.

Statistical analyses

Data were analyzed using SAS version 6.[30] Associations between endotoxin exposure and respiratory symptoms were evaluated by calculating Prevalence Rate Ratios[31,32] using Cox' proportional hazards model[33], as modified by Breslow[34] with the SAS PHREG procedure. Absolute across-shift peak flow changes were calculated by subtracting post-shift from pre-shift values. Relative changes were computed by dividing the absolute change by the age- and height-specific predicted value[9], and multiplying by 100%. Advantage of the latter approach is that comparisons of relative decreases and increases across the different work shifts can be made. Peak flow changes were averaged per worker for each shift to avoid dependence in subsequent statistical analyses. Relationships between endotoxin exposure and peak flow change were analyzed using linear regression analyses (PROC REG). Models were tested for homogeneity of the variance and for the presence and effects of outliers. Partial regression plots, residual plots and plots of Cook's D influence statistic versus independent variables were visually inspected.

Results

Respiratory health characteristics

In Table 6.1 characteristics of the 97 potato processing workers are shown. Ten percent of the workers indicated work-related obstructive symptoms. Respiratory health was similar in smokers and non-smokers although more non-smokers tended to be atopic. The two definitions of atopy were not in concordance for 22 workers (24%). Mean PEF was on average 5% and 6% higher than predicted in smokers (paired *t*-test; $P<0.05$) and non-smokers ($P<0.01$), respectively.

Table 6.1: Characteristics of 97 potato processing workers, stratified by current smoking.

	Smokers	Non-smokers
Number of subjects	31	66 *
Age (years)	42 ± 9 †	39 ± 9
Standing height (m)	1.79 ± 0.06	1.81 ± 0.06
Mean PEF (L/min)	592 ± 64	598 ± 72
Mean PEF (% of predicted‡	106 ± 11	105 ± 12
Work-related respiratory symptoms§	4 (13%)	6 (9%)
Chronic respiratory symptoms	7 (23%)	14 (21%)
Atopy definition A‖	8 (26%)	22 (35%)
Atopy definition B¶	9 (29%)	17 (27%)
Atopy definitions A and B	3 (10%)	14 (23%)

* Including 33 ex-smokers
† Arithmetic mean ± standard deviation
‡ According to the ERS[9]
§ Wheezing / Shortness of breath / Chest tightness ($n=31+65$)
‖ Specific IgE to ≥1 out of 5 common aero-allergens ($n=31+62$)
¶ Total IgE ≥100 Units/mL ($n=31+62$)

The PRR of work-related respiratory symptoms on ln-transformed endotoxin concentration was 2.3 (95%-confidence interval: 1.4 – 3.8). Over the interquartile range in endotoxin exposure of 249 to 1411 EU/m^3 an increased probability of 4.2 for work-related symptoms can be estimated. Adjustment for current smoking or atopic status in multiple models yielded a PRR close to 2.3 as well. This illustrates that smoking and atopy did not act as confounding variables in the relationship between endotoxin exposure and respiratory symptom prevalence. Stratification for smoking and atopic status yielded similar point estimates for subgroups, indicating that these host factors did not modify the studied association.

Peak flow measurements and variability

All 97 workers performed peak flow measurements for at least 16 days, while 71 of them completed all 23 days. There were no indications that peak flow patterns of the 26 workers who did not complete the entire study period were different from the 71 others. The mean CV between the three manoeuvres was on average 2.9% (range 0.9 – 9.1%). To investigate the presence of a learning effect, mean PEF was plotted versus measurement day. No increase of the mean PEF in the beginning of the study period could be observed.

In Table 6.2, Amp/Mean is shown for different time windows for different subgroups. Amp/Mean was highest during the morning shift, and lowest during leisure days. Differences between current smokers and non-smokers were not found. Atopics showed less peak flow variability than non-atopics, as indicated by the lower Amp/Mean values for all time windows.

Table 6.2: Amplitude/Mean ratio (%) calculated for Morning, Afternoon, Night shift and leisure days. Arithmetic means and 95%-confidence intervals are shown.

Subset	Morning shift	Afternoon shift	Night shift	Leisure days	Total
All observations	6.5*	5.4	5.9*	5.0	5.6
$n=93-97$	*(5.7–7.2)*	*(4.8–6.1)*	*(5.2–6.7)*	*(4.5–5.5)*	*(5.1–6.2)*
Current smokers	6.5	5.6	5.7	5.3	5.8
$n=30-31$	*(5.3–7.7*	*(4.8–6.4)*	*(4.5–7.0)*	*(4.3–6.2)*	*(4.9–6.8)*
Non-smokers	6.5	5.3	6.0	4.8	5.5
$n=63-66$	*(5.5–7.4)*	*(4.4–6.2)*	*(5.1–6.9)*	*(4.2–5.4)*	*(4.9–6.2)*
Atopics†	5.2	4.2	4.3	4.3	4.5
$n=17$	*(3.8–6.7)*	*(2.9–5.5)*	*(3.2–5.5)*	*(3.3–5.3)*	*(3.5–5.6)*
Non-atopics	6.7	5.7‡	6.2§	5.1	5.9‡
$n=73-76$	*(5.8–7.5)*	*(4.9–6.5)*	*(5.3–7.1)*	*(4.5–5.7)*	*(5.2–6.5)*

* Higher than on leisure days; $P<0.01$
† Both specific IgE to ≥1 out of 5 common aero-allergens and total IgE ≥100 Units/mL
‡ Higher than atopics; $P<0.1$
§ Higher than atopics; $P<0.05$

Across-shift peak flow changes

Eight-hour change in peak flow was available for 376 Morning shifts (93 workers), 367 Afternoon shift (93 workers), 319 Night shifts (85 workers) and 555 leisure days (93 workers). In Table 6.3 the mean relative PEF change is shown for different time windows for different subgroups. The mean peak flow increased across the Morning shift and decreased across both the Afternoon and Night shift. Across-shift change was similar in smokers and non-smokers, but tended to be smaller in atopics compared to non-atopics.

Table 6.3: Relative* change in PEF (%) across Morning, Afternoon and Night shift and leisure days (the latter measured at similar times as the Afternoon shift). Arithmetic means and 95%-confidence intervals are shown.

Subset	Morning shift	Afternoon shift	Night shift	Leisure days
All observations $n=85-93$	+2.7 *(+1.9; +3.5)*	−1.3 *(−1.9; −0.8)*	−1.7 *(−2.4; −0.9)*	−1.0 *(−1.5; −0.4)*
Work day 1 $n=77-87$	+3.1 *(+1.9; +4.4)*	−1.6 *(−2.4; −0.8)*	−2.2 *(−3.6; −0.8)*	—
Work days 2+3+4 $n=85-90$	+2.7 *(+1.9; +3.5)*	−1.1 *(−1.8; −0.5)*	−1.5 *(−2.3; −0.6)*	—
Current smokers $n=26-31$	+3.5 *(+2.3; +4.8)*	−1.1 *(−2.1; −0.2)*	−1.6 *(−2.8; −0.4)*	−1.4 *(−2.5; −0.4)*
Non-smokers $n=59-64$	+2.4 *(+1.3; +3.4)*	−1.4 *(−2.2; −0.7)*	−1.7 *(−2.7; −0.8)*	−0.7† *(−1.3; −0.1)*
Atopics‡ $n=12-17$	+0.9§ *(−0.8; +2.5)*	−1.0 *(−2.1; −0.0)*	−0.7 *(−2.1; +0.6)*	−0.8 *(−2.0; +0.3)*
Non-atopics $n=69-74$	+3.2 *(+2.3; +4.1)*	−1.3 *(−2.0; −0.7)*	−1.8 *(−2.7; −1.0)*	−1.0 *(−1.6; −0.3)*

* Change in PEF relative to age- and height-specific predicted value[9]
† Different from Afternoon shift (Student's paired *t*-test; $P<0.10$)
‡ Both specific IgE to ≥1 out of 5 common aero-allergens and total IgE ≥100 Units/mL
§ Different from Non-atopics (Student's two-sample *t*-test; $P<0.05$)

Relationships between endotoxin exposure and across-shift peak flow changes

Results of linear regression analyses for mean relative across-shift PEF change on ln-transformed endotoxin exposure are presented in Table 6.4. Overall negative coefficients indicate that endotoxin exposure was negatively associated with acute PEF changes. Inclusion of interaction terms in multiple models showed that coefficients for the Morning, Afternoon and Night shift were not significantly different from each other ($P>0.05$). In the last column multiple regression models with adjustment for shift are presented. Endotoxin exposure-response relationships tended to be steeper for the first work day after a leisure period.

Table 6.4: Relationships between endotoxin exposure and acute PEF changes.
Linear regression models for relative* across-shift change in PEF (%) on ln-transformed endotoxin exposure; coefficients and 95%-confidence intervals in brackets denote % change in PEF associated with an increase in endotoxin exposure from 249 to 1411 EU/m^3 (inter-quartile range).

Subset	Morning shift	Afternoon shift	Night shift	All work shifts†
All observations n=93/93/85	−0.44 *(−1.63;+0.74)*	−1.77‡ *(−2.57;−0.98)*	−0.79 *(−1.86;+0.28)*	−0.99 *(−1.58;−0.40)*
Work day 1 n=87/87/77	−1.82 *(−3.56;−0.07)*	−2.28 *(−3.41;−1.16)*	−0.93 *(−2.89;+1.03)*	−1.68 *(−2.61;−0.76)*
Work days 2+3+4 n=90/91/85	+0.04 *(−1.15;+1.23)*	−1.69 *(−2.60;−0.78)*	−0.82 *(−2.02;+0.38)*	−0.81 *(−1.44;−0.17)*
Current smokers n=29/30/26	−1.70 *(−3.34;−0.06)*	−1.01 *(−2.32;+0.31)*	+0.20 *(−1.51;+1.90)*	−0.86 *(−1.73;+0.02)*
Non-smokers n=64/63/59	+0.22 *(−1.35;+1.78)*	−2.24 *(−3.25;−1.22)*	−1.31 *(−2.69;+0.07)*	−1.07 *(−1.85;−0.30)*
Atopics§ n=17/17/12	−1.36 *(−6.75;+4.04)*	+0.06 *(−3.35;+3.47)*	−1.07 *(−5.15;+3.01)*	−0.78 *(−3.15;+1.59)*
Non-atopics n=72/73/69	−0.64 *(−1.93;+0.65)*	−1.85 *(−2.75;−0.94)*	−0.98 *(−2.22;+0.26)*	−1.15 *(−1.80;−0.49)*

* Change in PEF relative to age- and height-specific predicted value[9]
† Multiple regression models with adjustment for shift; numbers in these analyses are sums of the three numbers given in the first column.
‡ Coefficient different from Morning shift; $P<0.10$
§ Both specific IgE to ≥1 out of 5 common aero-allergens and total IgE ≥100 Units/mL

Stratification for current smoking and atopy yielded similar point estimates for subgroups, indicating that smoking and atopy did not modify the relationship between endotoxin exposure and peak flow change. Finally, adjustment for current smoking or atopic status in multiple regression analyses yielded similar coefficients as the crude estimates (results not shown). This shows that these host factors could not have confounded the relationships of interest.

Discussion

In this study, the prevalence of work-related respiratory symptoms and across-shift peak flow changes were associated with occupational endotoxin exposure among potato processing workers. Endotoxin-related peak flow change was most pronounced during the Afternoon shift (3 – 11 p.m. or 2 – 10 p.m.), and for all shifts larger on the first work day after a leisure period. Consistent differences in exposure-response relationships between smokers and non-smokers, and between atopics and non-atopics were not found.

In general, results of this study support the evidence for adverse respiratory effects of endotoxin exposure. In particular, this study focussed on influences of shift work and host factors on these effects. As could be expected because of the circadian rhythm, peak flow increased during the Morning shift and decreased during Afternoon and Night shifts.[16,17] The circadian rhythm has its top at about 4 p.m. and its minimum at about 4 a.m.[12] Even during consecutive days after rotating into the Night shift, the peak flow followed this pattern (Table 6.3). This suggests that the circadian rhythm did not change to a large extent after change of work shift. This is supported by the fact that the applied shift system in this industry appeared to be favorable for workers since small adaptions of the circadian rhythm were noted in studies in which different shift systems were compared.[35]

Amp/Mean was calculated to assess peak flow variability (Table 6.2). Neukirch *et al.*[28] found an average Amp/Mean of 9 – 12% in a study of 117 blue collar workers, aged 22 – 58 years, which is higher than in our study. In a general population study among 265 Dutch males from 20 – 70 years of age[27], Amp/Mean was 3 – 4%, which is somewhat lower than in our study. It has been suggested that a higher number of daily PEF readings increases the level of Amp/Mean.[36] Other studies reported a median Amp/Mean of 5%[25] and 8.5%[26] among healthy adults. Little is known about peak flow variability in shift workers. In our study, Amp/Mean was highest during the Morning shift, and lowest during the Afternoon shift. This can be explained by the first daily PEF measurement at 6 – 7 am, which is shortly after the minimum of the circadian rhythm, and this relatively low value results in a high variability[37], which is in agreement with results in Table 6.3. The influence of peak flow recording times

for different work shifts on the Amp/Mean was too large to use this parameter as a valid health outcome variable in our study. Therefore, across-shift changes were computed and related to exposure.

To our knowledge, only in one study different work shifts were considered in the study of endotoxin exposure-response relationships. Milton *et al.*[5] studied peak flow changes both during Morning shift and Night shift in a fiberglass manufacturing facility. For both shifts the same cut-off level of across-shift PEF change, −5%, was used in analyses. As expected because of the circadian rhythm, a larger number of workers showed a PEF decrease ≥5% during the Night shift compared to the Morning shift. The observed negative association between endotoxin exposure and peak flow change was similar for both types of work shifts. Results from our study support this finding. Very few other studies focussed on the influence of type of work shift on respiratory effects of occupational exposures. Pasker *et al.*[14] showed that differences in across-shift lung function change between zinc oxide exposed and non-exposed workers were larger in the Night shift, as compared to the day shift. More study is needed to evaluate the effect of type of work shift on exposure-related respiratory effects.

Endotoxin-related peak flow changes were largest on the first work day after a leisure period. This agrees with our previous study in this industry[20] using repeated spirometry across the Afternoon shift, and is also consistent with findings that endotoxin-induced symptoms were most severe on Mondays and became milder the following days.[38] The occurrence of a short-term adaption or 'tolerance' has been reviewed previously[39], and might play a role in the explanation of this phenomenon.

The influence of the host factors smoking and atopy was also evaluated in this study. Consistent differences between atopics and non-atopics could not be observed, which supports results from an experimental study.[6] Consistent differences between smokers and non-smokers could not be detected, either. A small controlled study[7] suggested that smokers showed a larger endotoxin-related response than non-smokers. However, only 17 smokers were compared to 11 non-smokers in the quoted study. It must be stressed that if our study had been limited to only one type of work shift, different conclusions with regard to interfering effects of the mentioned host factors could have been drawn (Table 6.4). A major implication of our findings is that results of studies among shift

workers may be misinterpreted if not all work shifts are being taken into account.

Some aspects of this study require further discussion. Firstly, repeated peak flow monitoring was used to measure acute respiratory effects. Workers performed the measurements themselves, hence no quality assurance was possible. One Canadian study[40] suggests that some workers investigated for occupational asthma had falsified their peak flow results. Visual inspection of peak flow-time graphs in our study showed no eye-catching patterns suggestive of falsification. Although falsification in our study cannot be excluded, it is not likely to play an important role since the purpose of our study was not the detection of occupational asthma, for which financial compensation is possible in Canada. In addition, in the quoted study workers had to record peak flow every two hours for on average 36 days, which is more demanding than the effort required in our study. Moreover, results in Table 6.3 indicate that patterns in peak flow change were consistent with expectations on the basis of the circadian rhythm.

It cannot be excluded that other bacterial components like peptidoglycan[41,42] play a part in observed peak flow changes since a previous study in this industry showed that endotoxin levels were related to bacterial counts.[19] It could be argued that endotoxin is a general marker for the presence of micro-organisms in organic dust in this industry.

We conclude that endotoxin exposure in the potato processing industry is related to across-shift peak flow changes and the occurrence of work-related respiratory symptoms, and is therefore likely to play a role in acute work-related respiratory effects. The host factors smoking and atopy are no important modifying factors in these relationships. In respiratory health studies among shift workers, it is important to investigate all work shifts before drawing definitive conclusions on exposure-response relationships. More study is needed to unravel the impact of possibly interfering factors in adverse effects of occupational endotoxin exposure.

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References

1. Castellan RM, Olenchock SA, Kinsley KB, Hankinson JL. Inhaled endotoxin and decreased spirometric values. An exposure-response relation for cotton dust. *N Eng J Med* 1987; 317: 605–610.
2. Rylander R, Bake B, Fischer JJ, Helander IM. Pulmonary function and symptoms after inhalation of endotoxin. *Am Rev Respir Dis* 1989; 140: 981–986.
3. Donham K, Haglind P, Peterson Y, Rylander R, Belin L. Environmental and health studies of farm workers in Swedish confinement buildings. *Br J Ind Med* 1989; 46: 31–37.
4. Smid T, Heederik D, Houba R, Quanjer PhH. Dust- and endotoxin related acute lung function changes and work related symptoms in the animal feed industry. *Am J Ind Med* 1994; 25: 877–888.
5. Milton DK, Wypij D, Kriebel D, Walters MD, Hammond K, Evans JS. Endotoxin exposure-response in a fiberglass manufacturing facility. *Am J Ind Med* 1996; 29: 3–13.
6. Blaski CA, Clapp WD, Thorne PS, Quinn TJ, Watt JL, Frees KL, Yagla SJ, Schwartz DA. The role of atopy in grain dust-induced airway disease. *Am J Respir Crit Care Med* 1996; 154: 334–340.
7. Haglind P, Rylander R. Exposure to cotton dust in an experimental cardroom. *Br J Ind Med* 1984; 41: 340–345.
8. Michel O. Human challenge studies with endotoxins. *Int J Occup Environ Health* 1997; 3: S18–S25.
9. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernoult JC. Lung volumes and forced ventilatory flows. *Eur Respir J* 1993; 6, Suppl. 16: 5–40.
10. Cinkotai FF, Sharpe TC, Gibbs ACC. Circadian rhythms in peak expiratory flow rate in workers exposed to cotton dust. *Thorax* 1984; 39: 759–765.
11. Hetzel MR, Clark TJH. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. *Thorax* 1980; 35: 732–738.
12. Eisen EA, Wegman DH, Kriebel D. Application of peak expiratory flow in epidemiologic studies of occupation. *Occup Med: State of the Art Reviews* 1993; 8: 265–277.
13. Sigsgaard T, Malmros P, Nersting L, Petersen C. Respiratory disorders and atopy in Danish refuse workers. *Am J Respir Crit Care Med* 1994; 149: 1407–1412.
14. Pasker HG, Peeters M, Genet P, Clément J, Nemery B, van de Woestijne KP. Short-term ventilatory effects in workers exposed to fumes containing zinc oxide: Comparison of forced oscillation technique with spirometry. *Eur Respir J* 1991; 10: 1523–1529.

15. Marquart H, Smid T, Heederik D, Visschers M. Lung function of welders of zinc-coated mild steel: Cross-sectional analysis and changes over five consecutive work shifts. *Am J Ind Med* 1989; 16: 289 – 296.
16. Walford J, Lammers B, Schilling RSF, Van den Hoven van Genderen D, Van der Veen YG. Diurnal variation in ventilatory capacity. An epidemiological study of cotton and other factory workers employed on shift work. *Br J Ind Med* 1966; 23: 142 – 148.
17. Love RG. Lung function studies before and after a work shift. *Br J Ind Med* 40: 153 – 159.
18. Dimich H.D., and T.D. Sterling. 1981. Ventilatory function changes over a work-shift. *Br J Ind Med* 38: 152 – 155.
19. Zock JP, Heederik D, Kromhout H. Exposure to dust, endotoxin and micro-organsims in the potato processing industry. *Ann Occup Hyg* 1995; 39: 841 – 854.
20. Zock, JP, Hollander A, Heederik D, Douwes J. Acute lung function changes and low endotoxin exposures in the potato processing industry. *Am J Ind Med* 1998; 33: 384 – 391.
21. Brederode D, Zock JP, Heederik D. Een vergelijking van verschillende methoden voor het analyseren van piekstroombepalingen. *T Soc Gezondheidsz* 1995; 73: 77 – 82. (In Dutch, with a summary in English.)
22. Biersteker K, Van Dijk WH, Eissens JBMF, Van Geuns HA. Ervaringen met geneeskundig onderzoek op CARA bij gemeentepersoneel te Rotterdam in 1970 – 1971. *T Soc Geneesk* 1974; 52: 158 – 162. (In Dutch, with a summary in English.)
23. Quanjer PhH, Lebowitz MD, Gregg I, Miller MR, Pedersen OF (eds). Peak expiratory flow. Conclusions and recommendations of a Working Party of the European Respiratory Society. 1995: 1 – 8.
24. Moscato G, Godnic-Cvar J, Maestrelli P, Malo JL, Burge PS. Statement on self-monitoring of peak expiratory flows in the investigation of occupational asthma. *Eur Respir J* 1995; 8: 1605 – 1610.
25. Quackenboss JJ, Lebowitz MD, Krzyzanowski M. The normal range of diurnal changes in peak expiratory flow rates. *Am Rev Respir Dis* 1991; 143: 323 – 330.
26. Higgins BG, Britton JR, Chinn S, Jones TD, Jenkinson D, Burney PGJ, Tattersfield. AE. The distribution of peak expiratory flow variability in a population sample. *Am Rev Respir Dis* 1989; 140: 1368 – 1372.
27. Boezen HM, Schouten JP, Postma DS, Rijcken B. Distribution of peak expiratory flow variability by age, gender and smoking habits in a random population sample aged 20 – 70 years. *Eur Respir J* 1994; 7: 1814 – 1820.
28. Neukirch F, Liard R, Segala C, Korobaeff M, Henry C, Cooreman J. Peak expiratory flow variability and bronchial responsiveness to methacholine: An epidemiologic study in 117 workers. *Am Rev Respir Dis* 1992; 146: 71 – 75.
29. Doekes G, Douwes J, Wouters I, De Wind S, Houba R, Hollander A. Enzyme immunoassaysfor total and allergen specific IgE in population studies. *Occup Environ Med* 1996; 53: 63 – 70.
30. SAS Institute Inc. SAS User's Guide, version 6. Cary, North Carolina: SAS, 1989.
31. Lee J, Chia KS. Estimation of prevalence rate ratios for cross sectional data: An example in occupational epidemiology. *Br J Ind Med* 1993; 50: 861 – 864.
32. Axelson O. Some recent developments in occupational epidemiology. *Scand J Work Environ Health* 1994; 20: 9 – 18.
33. Cox DR. Regression models and life-tables. *J Stat Soc B* 1972; 34: 187 – 220.

34. Breslow NE. Covariance analysis of censored survival data. *Biometrics* 1974; 30: 89–99.
35. Knauth P. Designing better shift systems. *Appl Ergonom* 1996; 27: 39–44.
36. Lebowitz MD, Krzyzanowski M, Quackenboss JJ, O'Rourke MK. Diurnal variation of PEF and its use in epidemiological studies. *Eur Respir J* 1997; 10: 49s–56s.
37. Venables KM, Davison AG, Browne K, Newman Taylor AJ. Pseudo-occupational asthma. *Thorax* 1989; 44: 760–761.
38. Rylander R, Imbus HR, Suh MW. Bacterial contamination of cotton as an indicator of respiratory effects among card room workers. *Br J Ind Med* 1979; 36: 299–304.
39. Ziegler-Heitbrock HWL, Blumenstein M, Käfferlein E, Kieper D, Petersmann I, Endres S, Flegel WA, Northoff H, Riethmüller G, Haas JG. *In vitro* desensitization to lipopolysaccharide suppresses tumor necrosis factor, interleukin-1 and interleukin-6 gene expression in a similar fashion. *Immunology* 1992; 75: 264–268.
40. Malo JL, Trudeau C, Ghezzo H, L'Archevêque J, Cartier A. Do subjects investigated for occupational asthma through serial peak expiratory flow measurements falsify their results? *J Allergy Clin Immunol* 1995; 96: 601–607.
41. Verhoef J, Kalter E. Endotoxic effects of peptidoglycan. In: Ten Cate JW, Büller HR, Sturk A (eds). Bacterial endotoxins: Structure, biomedical significance, and detection with the Limulus Amebocyte Lysate test. New York: Alan R. Liss, 1985: 101–112.
42. Burroughs M, Rozdzinski E, Geelen S, Tuomanen E. A structure-activity relationship for induction of meningeal inflammation by muramyl peptides. *J Clin Invest* 1993; 92: 297–302.

7. Evaluation of chronic respiratory effects in the potato processing industry: Indications of a Healthy Worker Effect?[1]

Abstract

Objectives: To investigate the occurrence of chronic respiratory effects of organic dust exposure in the potato processing industry.

Methods: Self-reported chronic respiratory symptoms and spirometric lung function were assessed in a cross-sectional study among 135 potato processing workers. A comprehensive study of current exposure to dust, endotoxin and potato antigens had been performed previously. Workers were grouped in low and high exposure categories for each of the three exposure indices. Relationships between exposure levels and respiratory health parameters were investigated either by calculating prevalence rate ratios or by performing linear regression analyses. Atopic status was determined by measuring total IgE and specific IgE to five common aero-allergens in workers' sera.

Results: Evident relationships between current exposure indices and respiratory health in the entire group were not observed. Workers employed ≤ 5 years showed a twofold higher prevalence rate of respiratory symptoms, a lower lung function and a higher endotoxin exposure compared with workers employed for more than 5 years. In addition, atopy was also more prevalent in workers employed ≤ 5 years. After stratification for employment duration no consistent relationships between exposure level and respiratory health parameters could be detected, either.

Conclusions: This study does not demonstrate chronic respiratory effects of organic dust exposure in the potato processing industry, despite the fact that endotoxin exposure levels observed in this industry have been reported to be associated with lung function decreases in other occupational settings. A likely explanation for not detecting apparent effects might be that a considerable number of symptomatic workers drops out of this industry within a few years after job commencement, suggesting a 'Healthy Worker Effect'.

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Introduction

In the potato processing industry high exposure levels of airborne dust, viable micro-organisms and endotoxin have been observed.[1-3] In the same studies a respiratory symptom prevalence of 16–46% has been reported, predominantly shortness of breath and chest tightness. Flu-like symptoms such as malaise and muscular pains, even leading to sick-leave, have been reported in departments with an exceptionally high microbial exposure. These respiratory and systemic disorders were consistent with the so called Organic Dust Toxic Syndrome (ODTS).[4-7] In our own surveys in the potato processing industry, a specific IgG response to airborne dust was observed, which appeared to be related to airborne antigen exposure.[8] A Type III allergic reaction, however, was not likely because this response was predominated by production of specific IgG_4 antibodies[9], which was not related with the presence of symptoms.

In the same studies bacterial endotoxin appeared to be an important constituent of the organic dust mixture[2,3], and significant associations between endotoxin exposure and across-shift lung function changes were demonstrated.[10] Thus it was concluded that endotoxin played a role in the etiology of acute respiratory effects, and the question arose whether the observed high endotoxin exposure levels might also be related to chronic respiratory health effects in this industry. Endotoxin-related chronic respiratory health effects have been described among cotton workers[11-13], pig farmers[14-16] and grain and animal feed workers.[17] Reported effects in the quoted studies consisted of increased prevalence of bronchitis-like symptoms, increased non-specific bronchial hyperresponsiveness, and decrements in FEV_1 and other lung function parameters. In several studies[11,16,17] weight-based organic dust exposure as such was less clearly related to respiratory health outcome than endotoxin exposure.

This report focuses on a cross-sectional analysis of chronic respiratory symptoms and respiratory function, and their relationships with dust, endotoxin and antigen exposure in four potato processing plants. A description of the production process and detailed information on exposure can be found elsewhere.[3,8]

Subjects and methods

Population and questionnaire

The study population comprised 142 male workers from four plants of a large potato processing company. This industry is characterised by a processing campaign from August to March. Consequently, workers are not exposed to organic dust during the other 4–5 months of the year. In this period, operators are employed in maintenance work which involves stripping down the plant and preparing it for the next campaign. A few percent of the workers is exclusively employed during the processing campaign. There is a considerable amount of job rotation from year to year.

Workers completed a self-administered questionnaire on occupational history in potato processing, smoking habits, and the Dutch version of a questionnaire on respiratory symptoms of the British Medical Research Council.[18] Number of pack-years[17] was calculated as a measure for cumulative smoking dose.

Lung function

Forced expiratory lung function measurements were performed by 132 workers just before or at the beginning of the potato processing campaign (July–August 1992). Most workers were tested at the start of the Afternoon shift. For practical reasons, however, 36 workers were tested after the morning shift, and 10 before the night shift.

Two Vicatest-5 dry rolling-seal spirometers (Jaeger, Breda, The Netherlands), calibrated before and after measurement sessions, were used by one of the authors and a trained assistant. The measurements and procedures including Body Temperature and Pressure Saturated with water vapour (BTPS) adjustments and data selection were performed according to the recommendations of the European Respiratory Society (ERS).[19] The following variables were registered: Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV_1), Maximal Mid-Expiratory Flow (MMEF) and Peak Expiratory Flow (PEF).

Atopic status

Blood serum samples were available from 125 workers; 109 workers were sampled both before and during the processing campaign.[8] Total IgE as well as specific IgE to five common aero-allergens (house dust mite, grass pollen, birch pollen, cat dander and dog dander) were determined in all 234 sera as described by Doekes *et al.*[20] Workers were considered atopic if specific IgE to at least one allergen was detected, either before or during the campaign. A second definition of atopy consisted of a total IgE concentration $\geq$100 Units/mL, either before or during the campaign.

Exposure assessment

Results of personal exposure measurements have been described elsewhere.[3,8] Briefly, 211 personal full-shift inhalable dust exposure measurements were performed among 128 workers. Bacterial endotoxin and potato antigens were determined in dust extracts of 195 and 186 filter halves, respectively. For all three exposure indices large differences existed between plants and between job categories. A job exposure matrix (JEM) based on a categorisation of job by plant with 27 categories was developed. The arithmetic mean exposure for each category was used as exposure estimate for workers in each group. Thus, for each of the 142 workers in this study dust, endotoxin and antigen exposure was assessed on the basis of his plant and job category. For the three exposure indices workers were divided in low and high exposure categories using median levels of each distribution arbitrarily as cut-off points. The use of current exposure estimates in chronic effect analyses might lead to misclassification due to the job rotation mentioned earlier. Therefore, employment duration in potato processing was also studied as a crude cumulative exposure index.

Statistical analyses

Data were analysed using SAS version 6.[21] Associations between exposure indices and respiratory symptoms were evaluated by calculating Prevalence Rate Ratios (PRRs).[22,23] Confidence intervals were calculated as proposed by Morris and Gardner.[24] Adjusted PRRs were calculated using Cox' proportional hazards model[25], as modified by Breslow[26] with the SAS PHREG procedure. Associations between exposure and lung function were evaluated using two-step linear regression analyses with age, standing height and number of pack-years smoked

as independent variables in the first step. Motivation for this approach was that employment duration, strongly associated with age, was one of the determining variables of interest. In classical multiple regressions analyses, colinearity between age and emplyment duration may lead to uninterpretable changes in coefficients, and can be avoided by a two-step analysis. Models were tested for homogeneity of the variance and for the presence and effects of outliers. Partial regression plots, residual plots and plots of Cook's *D* influence statistic versus independent variables were visually inspected. Interaction terms in multiple models were used to test differences in effect parameters between strata.

Results

Seven of the 142 workers (5%) had incomplete data on respiratory health or job history and were therefore excluded from analyses. Fifteen of the 132 workers who performed lung function tests (11%) did not meet the criteria for data selection of the ERS[20], two workers were not Caucasian, and two had incomplete questionnaires. All further lung function analyses were hence performed with data from 113 male Caucasian workers with complete data on respiratory health, smoking habits and job history.

In Table 7.1 demographic and respiratory health characteristics of the potato processing workers are presented. Results of separate questions on respiratory symptoms were combined because of small numbers and expected clustering. Asthma and bronchitis symptoms were prevalent in 12% and 9% of the workers, respectively. The majority of the workers had been employed for more than 10 years. The lung function variables FVC, FEV_1 and PEF were on average higher than age- and height-specific reference values[19], while the MMEF was on average 14% lower than predicted. Atopy was prevalent in about 30% of the workers for both definitions. In 92 of the 125 workers (74%) the two definitions of atopy were in concordance.

Table 7.1: Respiratory health characteristics of 135 potato processing workers

	n (%)	MEAN	RANGE
Age (years)		40.1	22 – 58
Employment in potato processing (years)		13.2	0 – 38
> 5 years employed	103 (76%)		
> 10 years employed	84 (62%)		
Smokers	56 (41%)		
Ex-smokers	41 (30%)		
Asthma symptoms*	16 (12%)		
Frequent wheezing	10 (7%)		
Chest tightness	12 (9%)		
Bronchitis symptoms†	12 (9%)		
Chronic cough	5 (4%)		
Chronic phlegm	5 (4%)		
Shortness of breath	7 (5%)		
Atopy definition A‡ (*N* = 125)	39 (31%)		
Atopy definition B§ (*N* = 125)	38 (30%)		
FVC‖		108¶	79 – 146
FEV_1		103¶	66 – 140
MMEF		85¶	28 – 174
PEF		121¶	64 – 189

* Frequent wheezing / chest tightness
† Chronic cough / chronic phlegm / shortness of breath
‡ Specific IgE to ≥ 1 out of 5 common aero-allergens
§ Total IgE ≥ 100 Units/mL
‖ Percentage of age- and height-specific reference value[19] (*N* = 113)
¶ $P < 0.01$ (Student's paired *t*-test)

Descriptive statistics of the distributions of estimated dust, endotoxin and antigen exposure in 135 workers in Table 7.2 indicate large ranges across the 27 categories. Estimated dust exposure was not correlated with endotoxin exposure (Pearson's R between logarithms was -0.11; $P>0.2$). Antigen exposure was significantly ($P<0.01$) correlated with both dust ($R=0.24$) and endotoxin exposure ($R=0.50$). For each exposure index, workers were grouped into 'low' and 'high' categories using 50-percentiles as cut-off points. There was a fair concordance of endotoxin and antigen exposure categories ($\chi^2=8.9$; $P<0.01$); 63% of the workers were placed in the same categories of endotoxin and antigen exposure.

Table 7.2: Descriptive statistics of estimated* personal exposure to dust, endotoxin and antigens among 135 potato processing workers

Exposure	AM	GM	Range	Median
Dust (mg/m^3)	2.93	1.61	0.2 – 29	1.55
Endotoxin (EU/m^3)	990	449	23 – 8167	406
Antigens (10^3 RAU/m^3)	10.7	2.48	0.04 – 170	1.79

* Each worker was given the arithmetic mean exposure of his plant specific job category.[3,8] Twenty-seven categories were distinguished.
AM Arithmetic Mean
GM Geometric Mean
EU Endotoxin Units
RAU Relative Antigen Units

Table 7.3 shows relationships between respiratory symptom prevalence, potential confounders and exposure proxies. Level of current exposure as well as employment duration were considered. Crude Prevalence Rate Ratios (PRRs) are presented for both asthma and bronchitis symptoms. Although confidence intervals were wide, asthma symptoms were more prevalent in smokers, while bronchitis symptoms were more prevalent in ex-smokers. In general, no consistent relationships with current exposure level were observed. Bronchitis symptoms however tended to be less prevalent in workers exposed to high dust concentrations. The most remarkable finding was that respiratory symptoms were less prevalent in workers who had been employed for a longer period in

potato processing. This seemed to be true for both asthma and bronchitis symptoms.

Table 7.3: Relationships between respiratory symptoms and possible determining variables. Crude prevalence rate ratios with 95%-confidence intervals for smoking, atopy, exposure indices and employment duration. $N = 135$

	Asthma symptoms*	Bronchitis symptoms†
Smokers‡	2.26 *(0.67–7.7)*	1.02 *(0.18–5.8)*
Ex-smokers‡	0.93 *(0.20–4.3)*	3.24 *(0.72–15)*
Atopy definition A§	1.65 *(0.62–4.4)*	1.84 *(0.60–5.7)*
Atopy definition B‖	1.27 *(0.46–3.5)*	0.86 *(0.24–3.1)*
High dust exposure	0.81 *(0.32–2.1)*	0.35 *(0.10–1.2)*
High endotoxin exposure	1.79 *(0.69–4.7)*	0.54 *(0.17–1.7)*
High antigen exposure	0.94 *(0.37–2.4)*	1.21 *(0.41–3.6)*
Employment duration >5 years	0.40 *(0.16–0.99)*	0.62 *(0.20–1.9)*
Employment duration >12 years¶	0.77 *(0.30–1.9)*	0.33 *(0.09–1.2)*

* Frequent wheezing / chest tightness
† Chronic cough / chronic phlegm / shortness of breath
‡ Never-smokers were taken as reference group
§ Specific IgE to ≥1 out of 5 common aero-allergens
‖ Total IgE ≥100 Units/mL
¶ Median

Linear regression models using age, standing height and number of pack-years for lung function variables showed an explained variance (R^2) between 11% (PEF) and 52% (FVC and FEV_1). Unstandardised residuals were used to study associations with potential confounders and exposure proxies. Workers who were tested after the work shift showed nonsignificant lower residuals compared with workers tested before the shift. As shown in Table 7.4, no consistent relationships between current exposure and lung function could be noted. The Table, however, does indicate that workers employed >5 years had higher lung function values than shorter employed workers. This difference was statistically significant for the peak flow, where a mean difference of 9% was observed. For

Table 7.4: Single linear regression coefficients with standard errors for atopy, exposure indices and employment duration on lung function residuals (standardised for age, standing height and number of pack-years smoked). Low exposure categories and short employment duration were taken as reference. $N=113$

	LUNG FUNCTION VARIABLE: L(/s)			
	FVC	FEV_1	MMEF	PEF
Atopy definition A* ($N=105$)	+0.12 (0.13)	+0.08 (0.11)	+0.06 (0.24)	−0.29 (0.47)
Atopy definition B† ($N=105$)	+0.07 (0.13)	+0.05 (0.11)	+0.09 (0.24)	+0.05 (0.46)
High dust exposure	−0.06 (0.11)	+0.02 (0.10)	+0.09 (0.22)	+0.16 (0.41)
High endotoxin exposure	+0.16 (0.11)	+0.02 (0.10)	−0.26 (0.21)	−0.07 (0.41)
High antigen exposure	+0.02 (0.11)	−0.03 (0.10)	−0.15 (0.22)	−0.15 (0.41)
Employment duration >5 years	+0.12 (0.13)	+0.16 (0.12)	+0.31 (0.26)	+1.02 (0.49) §
Employment duration >12 years‡	+0.07 (0.11)	+0.08 (0.10)	+0.10 (0.22)	+0.31 (0.41)

* Specific IgE to ≥1 out of 5 common aero-allergens
† Total IgE ≥100 Units/mL
‡ Median
§ $P<0.05$

all parameters, lung function expressed as percentage of the reference value was on average higher in workers employed >5 years. This difference was statistically significant for the PEF ($P<0.05$) and borderline significant for the FVC ($P<0.1$).

Because employment duration was apparently related to respiratory health outcome, it was investigated whether possible determinants were also related to employment duration. Endotoxin exposure was on average twofold higher among workers employed ≤5 years compared to workers employed >5 years (*t*-test; $P<0.01$). As a result, 63% of the workers employed ≤5 years were classified in the high endotoxin exposure group, against 44% of the workers employed >5 years. Differences in dust and antigen exposure between the two groups were smaller than observed for endotoxin.

With regard to atopic status, prevalence of specific IgE to common allergens was 56% among workers employed ≤5 years and 24% among workers employed >5 years (Fisher-Exact test; $P<0.01$). A higher prevalence of atopy in workers employed ≤5 years was also noted when considering different age tertiles. A smaller difference was observed for total IgE; 41% of the shortly employed workers showed a total IgE concentration above 100 Units/mL, against 28% of the longer employed workers ($P>0.1$).

To account for the observed differences between shortly and longer employed workers, further analyses were performed after stratification for employment duration. To avoid too small numbers, the median employment duration (12 years) was used arbitrarily as cut-off point. In Table 7.5 relationships between exposure indices and respiratory symptoms are shown in the two strata. In general, no consistent exposure-related effects could be observed and statistically significant differences in risk estimates were not found. After adjusting for smoking in multiple models, PRRs did not change essentially. It was however notable that within the subset of workers employed >12 years, no bronchitis symptoms were reported among workers exposed to high dust or endotoxin levels.

Table 7.6 shows regression coefficients of exposure indices on lung function variables after stratification for employment duration. In the subset of workers employed ≤12 years nearly all point estimates were negative, and in the subset of workers employed >12 years the majority was positive. However, coefficients were close to zero and, with one exception, not statistically significant.

Table 7.5: Relationships between respiratory symptoms and possible determining variables after stratification for employment duration. Crude prevalence rate ratios with 95%-confidence intervals for high versus low exposure.

	≤12 years employed ($n=67$)		>12 years employed ($n=68$)	
	Asthma symptoms*	Bronchitis symptoms†	Asthma symptoms	Bronchitis symptoms
High dust exposure	1.43 *(0.42–4.8)*	0.90 *(0.25–3.3)*	0.46 *(0.11–1.9)*	0‡
High endotoxin exposure	2.84 *(0.64–13)*	0.65 *(0.19–2.2)*	1.07 *(0.26–4.4)*	0‡
High antigen exposure	0.99 *(0.29–3.4)*	1.54 *(0.45–5.2)*	0.90 *(0.22–3.7)*	0.60 *(0.06–6.3)*

* Frequent wheezing / chest tightness
† Chronic cough / chronic phlegm / shortness of breath
‡ No cases in high exposure category

Table 7.6: Single linear regression coefficients with standard errors for exposure indices on lung function residuals (standardised for age, standing height and number of pack-years smoked) after stratification for employment duration. Low exposure categories were taken as reference.

≤12 years employed ($n=55$)

	LUNG FUNCTION VARIABLE: L(/s)			
	FVC	FEV_1	MMEF	PEF
High dust exposure	−0.27 (0.15)	−0.12 (0.14)	−0.03 (0.33)	+0.17 (0.65)
High endotoxin exposure	−0.05 (0.14)	−0.14 (0.13)	−0.31 (0.30)	−0.49 (0.60)
High antigen exposure	−0.13 (0.14)	−0.13 (0.13)	−0.17 (0.31)	−0.33 (0.61)

>12 years employed ($n=58$)

	LUNG FUNCTION VARIABLE: L(/s)			
	FVC	FEV_1	MMEF	PEF
High dust exposure	+0.08 (0.18)	+0.10 (0.15)	+0.15 (0.32)	−0.01 (0.58)
High endotoxin exposure	+0.38 (0.16) * †	+0.19 (0.15) ‡	−0.20 (0.31)	+0.38 (0.56)
High antigen exposure	+0.16 (0.17)	+0.06 (0.15)	−0.13 (0.31)	−0.01 (0.56)

* $P<0.05$
† Coefficient different from group of workers employed ≤12 years; $P<0.05$
‡ Coefficient different from group of workers employed ≤12 years; $P<0.10$

Discussion

In this study respiratory health of potato processing workers in relation to organic dust exposure was investigated. Asthma symptoms, defined as frequent wheezing and/or chest tightness, were more prevalent in this study than among a large sample of office workers[27] and among other non-exposed controls[17] where the same questionnaire was used. Prevalence of chest tightness appeared to be higher than observed among animal feed workers[17], but was similar to that in pig farmers.[14,28] Both groups are occupationally exposed to organic dust. Endotoxin is a recognised risk factor for chronic bronchitis.[11,13,29] Nevertheless, the prevalence of chronic cough, indicative of bronchitis, was lower in comparison with the other mentioned populations. Lung function was on average not lower than expected. Percentages of reference values were more or less similar to those observed among pig farmers[16] of whom a similar percentage of smokers but clearly more symptomatics (48%).

Three indices of organic dust exposure were considered: weight-based dust, endotoxin and antigens. In a previous study we showed that a specific IgG_4 response, which appeared to be related to airborne antigen exposure, was not associated with the presence of respiratory symptoms.[8] Thus, an adverse respiratory effect of antigen exposure was *a priori* not likely. This was confirmed in the present study by the absence of apparent relationships between antigen exposure and respiratory health outcome.

There is strong evidence that endotoxin has acute and chronic health effects such as lung function decrease and increased bronchial reactivity at levels encountered in this study. The levels found in this industry have been reported to be clearly associated with lung function decreases in several other occupational settings.[11,13–17] Contrary to expected, we did not observe clear cut chronic respiratory effects of endotoxin exposure in our study. Dust exposure was low in most departments of the studied plants.[3] Moreover, in several studies among organic dust exposed workers it was shown that dust exposure was less clearly related to chronic respiratory symptoms and lung function than endotoxin exposure.[15–17]

Some reasons for not detecting relationships can be considered. An important issue is the seasonal pattern of organic dust exposure in this industry. This pattern is present in a few other, mostly agriculture-related, industries with

campaign-work such as sugar beet refinement.[30] Little is known about chronic effects due to occupational exposures in these type of industries. It can be speculated that a few months 'recovery time' counters irreversible effects of organic dust exposure.

Secondly, a number of lung function measurements was performed after the morning shift. Since endotoxin exposure can cause acute lung function changes, chronic effects might have been obscured by acute effects. However, apparent adverse effects on lung function were not noted, implicating that the occurrence of acute effects did not strongly influence our lung function data.

The third problem in the design of our study might be the use of current exposure estimates for chronic effect analyses. When regarding employment duration as a crude cumulative exposure index, an interfering effect seemed present because workers who had been employed for more than five years had less respiratory symptoms, a higher lung function and less atopy. This apparent selection is suggestive of the 'Healthy Worker Effect', as reported mostly in longitudinal studies.[31,32] It can be speculated that symptomatic workers drop out of this industry after a few years. In addition, a trend of lower endotoxin exposures in longer employed workers was observed, suggesting that job rotation also plays a role in this process. However, longitudinal data on respiratory health and employment in the potato processing industry are required to support this hypothesis. Unfortunately, our study in the potato processing industry has been ongoing for only a few years, and does not allow a proper investigation into drop-out of workers.

Bronchitis symptoms were less prevalent in workers with a high dust exposure, particularly in workers employed for more than 12 years. A similar finding for chronic phlegm has been reported previously in a study among animal feed workers.[17] A possible explanation is that workers reporting cough and phlegm rotate into jobs with a lower (visible) dust exposure. Apart from the suggested drop-out of symptomatic workers, this is suggestive of another health-related selection.[33] In contrast to dust, it is mostly less obvious which jobs involve high endotoxin exposures.

Prevalence of atopy, defined as specific IgE to one or more out of five common aero-allergens, amounted 31%. This is in concordance with 32% reported in a Dutch general population study among 1286 males, aged 20 – 70 years.[34] Atopy was twofold more prevalent in workers employed $\leq$5 years. This corrobo-

rates the health-related selection mentioned earlier. From population studies it is known that prevalence of atopy decreases with increasing age.[34,35] The difference in age, however, could only partly explain the difference in atopic prevalence between shortly and longer employed workers. Interestingly, these findings are consistent with a longitudinal study among grain workers[36] in which atopics developed more respiratory symptoms after commencement of employment, and more atopics than non-atopics dropped out of the grain industry.

Total IgE is less clearly related with age[34,35,37], and elevated total IgE actually showed a smaller difference between shortly and longer employed workers. Associations between atopic status and asthma and/or FEV_1 have been reported previously[38,39], but were not observed in our population. The suggested selection probably also played an interfering role here.

We conclude that no chronic respiratory effects of organic dust exposure in the current workforce in the potato processing industry have been observed. Several observations within our study, however, strongly suggest that this might be at least partially due to the fact that symptomatic workers drop out of this industry within a few years after job commencement.

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References

1. Dutkiewicz J. Bacteria, fungi and endotoxin as potential agents of occupational hazard in a potato processing plant. *Am J Ind Med* 1994; 25: 43–46.
2. Hollander A, Heederik D, Kauffman H. Acute respiratory effects in the potato processing industry due to a bio-aerosol exposure. *Occup Environ Med* 1994; 51: 73–78.
3. Zock JP, Heederik D, Kromhout H. Exposure to dust, endotoxin and micro-organisms in the potato processing industry. *Ann Occup Hyg* 1995; 39: 841–854.
4. doPico GA. Report on diseases. *Am J Ind Med* 1986; 10: 261–265.

5. Donham KJ, Thorne PS. Agents in organic dust: criteria for a causal relationship. *Am J Ind Med* 1994; 25: 33–39.
6. Rask-Andersen A. Organic dust toxic syndrome among farmers. *Br J Ind Med* 1989; 46: 233–238.
7. Richerson HB. Unifying concepts underlying the effect of organic dust exposures. *Am J Ind Med* 1990; 17: 139–142.
8. Zock JP, Doekes G, Heederik D, van Zuylen M, Wielaard P. Airborne dust antigen exposure and specific IgG response in the potato processing industry. *Clin Exp Allergy* 1996; 26: 542–548.
9. Van der Zee JS, Aalberse RC. The role of IgG. In: Lessof MH, Lee TH, Kemeny DM (eds). Allergy: An international textbook. Chichester: John Wiley & Sons, 1987: 49–67.
10. Zock JP, Hollander A, Heederik D, Douwes J. Acute lung function changes and low endotoxin exposures in the potato processing industry. *Am J Ind Med* 1998 (in press).
11. Kennedy SM, Christiani DC, Eisen EA, Wegman DH, Greaves IA, Olenchock SA, Ting-Ting Y, Pei-Lian L. Cotton dust and endotoxin exposure-response relationships in cotton textile workers. *Am Rev Respir Dis* 1987; 135: 194–200.
12. Rylander R, Bergström R. Bronchial reactivity among cotton workers in relation to dust and endotoxin exposure. *Ann Occup Hyg* 1993; 37: 57–63.
13. Sigsgaard T, Pedersen OF, Juul S, Gravesen S. Respiratory disorders and atopy in cotton, wool and other textile mill workers in Denmark. *Am J Ind Med* 1992; 22: 163–184.
14. Heederik D, Brouwer R, Biersteker K, Boleij JSM. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. *Int Arch Occup Environ Health* 1991; 62: 595–601.
15. Zejda JE, Barber E, Dosman JA, Olenchock SA, McDuffie HH, Rhodes DVM, Hurst T. Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. *J Occup Med* 1994; 36: 49–56.
16. Preller L, Heederik D, Boleij JSM, Vogelzang PFJ, Tielen MJM. Lung function and chronic respiratory symptoms of pig farmers: focus on exposure to endotoxins and ammonia and use of disinfectants. *Occup Environ Med* 1995; 52: 654–660.
17. Smid T, Heederik D, Houba R, Quanjer PhH. Dust- and endotoxin-related respiratory effects in the animal feed industry. *Am Rev Respir Dis* 1992; 146: 1474–1479.
18. Biersteker K, Dijk WH van, Eissens JBMF, Geuns HA van. Ervaringen met geneeskundig onderzoek op CARA bij gemeentepersoneel te Rotterdam in 1970– 1971. *T Soc Geneesk* 1974; 52: 158–162. (In Dutch, with a summary in English.)
19. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernoult JC. Lung volumes and forced ventilatory flows. *Eur Respir J* 1993; 6, Suppl. 16: 5–40.
20. Doekes G, Douwes J, Wouters I, de Wind S, Houba R, Hollander A. Enzyme immunoassaysfor total and allergen specific IgE in population studies. *Occup Environ Med* 1996; 53: 63–70.
21. SAS Institute Inc. SAS User's Guide, version 6. Cary, North Carolina: SAS, 1989.
22. Lee J, Chia KS. Estimation of prevalence rate ratios for cross sectional data: An example in occupational epidemiology. *Br J Ind Med* 1993; 50: 861–864.
23. Axelson O. Some recent developments in occupational epidemiology. *Scand J Work Environ Health* 1994; 20: 9–18.
24. Morris JA, Gardner MJ. Calculating confidence intervals for relative risks (odds ratios) and standardised ratios and rates. *Br Med J* 1988; 296: 1313–1316.

25. Cox DR. Regression models and life-tables. *J Stat Soc B* 1972; 34: 187–220.
26. Breslow NE. Covariance analysis of censored survival data. *Biometrics* 1974; 30: 89–99.
27. Preller L, Zweers T, Brunekreef B, Boleij JSM. Gezondheidsklachten en klachten over het binnenklimaat in kantoorgebouwen. Rapport S–83. Den Haag: DGA, Ministerie van Sociale Zaken, 1990. (In Dutch.)
28. Bongers P, Houthuijs D, Remijn B, Brouwer R, Biersteker K. Lung function and respiratory symptoms in pig farmers. *Br J Ind Med* 1987; 44: 819–823.
29. Jacobs RR. Airborne endotoxins: An association with occupational lung disease. *Appl Ind Hyg* 1989; 4: 50–56.
30. Forster HW, Crook B, Platts BW, Lacey J, Topping MD. Investigation of organic aerosols generated during sugar beet slicing. *Am Ind Hyg Assoc J* 1989; 50: 44–50.
31. Eisen EA, Wegman DH, Louis TA, Smith TJ, Peters JM. Healthy worker effect in a longitudinal study of one-second forced expiratory volume (FEV_1) and chronic exposure to granite dust. *Int J Epidemiol* 1995; 24: 1154–1162.
32. Broder I, Corey P, Davies G, Hutcheon M, Mintz S, Inouye T, Hyland R, Leznoff A, Thomas P. Longitudinal study of grain elevator and control workers with demonstration of the healthy worker effect. *J Occup Med* 1985; 27: 873–880.
33. Checkoway H, Pearce N, Crawford-Brown DJ. Research methods in occupational epidemiology. New York: Oxford University Press, 1989.
34. Kerkhof M, Droste JHJ, de Monchy JGR, Schouten JP, Rijcken B. Distribution of total serum IgE and specific IgE to common aeroallergens by sex and age, and their relationship to each other in a random sample of the Dutch general population aged 20–70 years. *Allergy* 1996; 51: 770–776.
35. Omenaas E, Bakke P, Elsayed S, Hanoa R, Gulsvik A. Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. *Clin Exp Allergy* 1994; 24: 530–539.
36. Dosman JA, McDuffie HH, Pahwa P. Atopic status as a factor in job decision making in grain workers. *J Occup Med* 1991; 9: 1007–1010.
37. Grigoreas C, Pappas D, Galatas ID, Kollias G, Papadimos S, Papadakis P. Serum total IgE levels in a representative sample of a Greek population. *Allergy* 1993; 48: 142–146.
38. Sunyer J, Antó JM, Castellsagué J, Soriano JB, Roca J, and the Spanish group of the European Study of Asthma. Total serum IgE is associated with asthma indepently of specific IgE levels. *Eur Respir J* 1996; 9: 1880–1884.
39. Omenaas E, Bakke P, Eide GE, Elsayed S, Gulsvik A. Total serum IgE and FEV_1 by respiratory symptoms and obstructive lung disease in adults of a Norwegian community. *Clin Exp Alllergy* 1995; 25: 682–689.

8. General discussion

Introduction

Aims of this study were (1) to quantify the occurrence of work-related respiratory health effects in potato processing workers, and (2) to study possible mechanisms. In this chapter the main findings, study design and applied methods of the study are discussed. Furthermore, implications inside and outside the potato processing industry are discussed, and possibilities for control measures are elaborated. Finally, the conclusions are summarised and objectives for further studies are suggested.

Main findings

Organic dust exposure assessment

The objective of exposure assessment was twofold: to characterise exposure to different organic dust constituents, and to distinguish homogeneous exposure groups of workers for epidemiological analyses.

Personal exposure to inhalable dust differed strongly between plants and between job categories. High dust exposure levels were strongly related to a few jobs in which workers were involved in handling dried starch or protein; this occurred on drying, sacking, expedition and storage departments. This confirmed results of previous studies in potato processing plants.[1,2] Both starch and protein dust consisted of relatively large particulates; the respirable dust fraction accounted for only 2 – 4 mass % of the inhalable dust concentration. Additional measurements with an Andersen 1 CFM Ambient Sampler (Andersen samplers Inc., Atlanta, GA, USA) and a Grimm 1.102 direct reading monitor[3] confirmed these findings, both for starch and for protein dust; Mean Median Aerodynamic Diameter was >10 μm. This indicates that it is not likely that large amounts of fine particles will deposit in the alveolar region. Nevertheless, the levels of inhalable nuisance dust exceeded the Dutch exposure standard of 10 mg/m^3 and were therefore unacceptably high in some of the departments.

The endotoxin exposure distribution showed a wide range across plants and job categories, but levels were overall high when compared to other occupational settings.[4,5] In the preliminary study in one of the plants[1], endotoxin expo-

sure levels were roughly tenfold lower. This is probably caused by the Tween-addition in extraction procedures in our later study.[6] In a Polish potato processing plant[2] endotoxin exposure levels were 100-fold higher than in our study. Although the analytical technique was similar (LAL test), other filters were used for sampling, while information on elution procedures was not available. This makes a comparison of endotoxin levels between both studies difficult. Moreover, in the Polish plant different process techniques were used; potatoes were peeled and subsequently steamed and blanched. After these steps, a dramatic raise in endotoxin levels to $>10^7$ EU/m^3 was observed.

Endotoxin exposure in our study was not associated with dust exposure; Pearson's R calculated with the log-transformed personal concentrations was 0.03. A drawback of the dust measurement technique in our study was that only dry matter was weighed, while a large amount of the aerosols in humid departments probably consisted of water. Relative humidity was measured as a crude proxy for exposure to humid aerosols. Endotoxin concentration tended to be higher in departments with a high relative humidity. Temperature also seemed to be an important factor (Fig. 2.2); a higher process water temperature in the fibre dehydration machinery was associated with higher levels of airborne bacteria and endotoxin. It was suggested that recycling of process water in this industry constitutes an important opportunity for bacterial growth and hence comprises an endotoxin source. This has also been observed in other industries with process water recycling systems.[7–10]

High airborne bacterial counts ($>10^5$ CFU/m^3) were observed in many departments, and bacterial counts were correlated with endotoxin exposure levels. This suggests that a steady growth of bacteria, and with that endotoxin release in process water, leads to simultaneous airborne exposure to viable bacteria and endotoxin. An unpublished study in the washing department of one of the plants showed that soil particles on the potatoes were the most important source of bacteria and endotoxin in wash water. This supports findings of a study in a sugarbeet processing plant[9], where bacterial counts were dominated by common soil-borne species.

Levels of airborne fungi were not extremely high: concentrations ranged from 200 to 15,000 CFU/m^3. This corroborates results of the Polish study.[2] Significant correlations between fungal counts and levels of any of the other exposure indices were not found. This indicates that fungi originate from different sources

and require different environmental conditions, such as another optimal temperature, different nutrients and aerobe circumstances.

For all micro-organisms it could be argued that the use of viable sampling techniques has drawbacks, such as a short sampling time resulting in 'snap-shot' measurements, and neglect of non-viable organic dust components. Endotoxin seemed to be a reasonable non-viable marker for Gram-negative bacteria.

Grouping of workers for exposure-response analyses

Since both personal dust and endotoxin exposure concentrations were largely determined by plant and job category, a Job Exposure Matrix (JEM) based on a categorisation of job by plant with 27 categories was developed. The arithmetic mean dust and endotoxin exposure for each category was used as exposure estimate for workers in each group. This classification yielded a between-group relative to within-group variance or 'contrast'[11,12] of 0.38, which was higher than on the basis of plant or job category only. Especially for endotoxin, twelve job categories and four plants could not be considered as homogenous exposure groups since a relatively high variability between workers within these groups was observed ($_{BW}\hat{R}_{.95} > 4$).[13] This leads to less power to detect exposure-response relationships, but not necessarily to attenuation towards the null.[12] An advantage of our approach was that not only the workers who had participated in the exposure monitoring program could be included in epidemiological analyses. Overall within-worker variance in endotoxin exposure was estimated at 0.54 (GSD = 2.1; range 1.1 to 3.2), which was probably underestimated because repeated measurements were performed on two consecutive work days.[14,15] Nevertheless, it suggested that for many job categories endotoxin exposure of a certain worker was similar for consecutive work shifts, probably due to the fact that working tasks did not vary to a large extent from day-to-day.

The study described in Chapter 5 was based on separate exposure measurements in one of the plants in another year. Two homogeneous exposure groups could be defined on the basis of job titles. The use of job title mean endotoxin concentrations in linear regression analyses did not change exposure-response relationships; the estimated contrast was optimal due to a low within-group between-worker variance. Endotoxin concentrations in this sub-study are not directly comparable to results of later measurements as described in Chapter 2, since no Tween-20 was used in elution procedures. An experimental study

performed in this industry showed that addition of Tween to the elution medium pyrogen-free water, yielded a sevenfold higher endotoxin concentration.[6]

Work-related allergies

The occurrence of an occupational Type I allergy was investigated by specific IgE measurements against airborne dust extract in workers' sera. Specific IgE against airborne dust extract could not be detected, supporting preliminary findings in this industry.[1] The quoted study also showed that IgG_4-reactions to this extract could fully be inhibited by potato extract, which was supported by findings in our study (Chapter 4). In combination with the suggestion that IgG_1 and IgG_4 antibodies to potato were directed against the same components as specific IgE[16], this indicated that our measurements of specific IgE to airborne dust extract provided a valid test for Type I potato allergy. In combination with the observation that a Type I allergy to potato is uncommon.[16,17], it can be concluded that a work-related Type I allergy is highly unlikely to play a role in explaining the etiology of work-related respiratory effects. It can, however, not be excluded that occasionally a worker might develop an allergy to potato. It can be expected that high airborne antigen levels in the plants can easily provoke severe allergic symptoms, making it likely that this worker drops out of this industry.

It has been described previously that potato proteins show IgE cross-reactivity with proteins from pollens.[18,19] In a study among 238 patients allergic to at least one out of three pollen allergens, 29% had detectable IgE against potato.[18] In the quoted study, specific IgE to potato could not be detected in sera from 91 subjects who were not allergic to pollens. In our study, elevated specific IgE to birch and/or grass pollen allergens was observed in 23 out of 131 workers (18%), but no specific IgE against potato could be found. In Table 6.4, no different endotoxin exposure-response relationships for atopics and non-atopics could be observed, and additional analyses showed that the exposure-response relationship for workers with detectable IgE against pollen was also similar.

Despite the absence of work-related IgE antibodies, a strong humoral immune response to airborne dust antigens was demonstrated. Specific IgG was found in nearly all workers, and the IgG_4 subclass was found in about half of the workers. Control subjects with no history in potato processing work, and newly hired workers had low specific IgG levels and no detectable specific IgG_4. IgG titres increased during the processing campaign, and this increase was dependent on

the antigen exposure level. Inhibition tests with potato extract and experiments with extracts after heat treatment revealed that responsible antigens were most probably heat-labile potato proteins.

No association was found between specific work-related IgG and the prevalence of work-related respiratory symptoms. Moreover, both the difference in IgG titres between the potato processing workers and the non-exposed control group, and the exposure-related increase in specific IgG titres seemed to be mainly due to specific antibodies of the IgG_4 subclass. IgG_4 antibodies are neither able to react as precipitating antibodies in Type III allergy nor to activate the complement system.[20] On the basis of these findings it is therefore unlikely that a Type III allergy and with that Hypersensitivity Pneumonitis plays a predominant role in the etiology of work-related respiratory effects. Dutkiewicz *et al.*[2] found that 40 out of 61 Polish potato processing workers had precipitins to microbial antigens present in the working environment. The presence of precipitins to some fungal species was significantly correlated with the occurrence of work-related symptoms, suggesting that Type III allergy against fungi might play a role in the etiology of work-related respiratory effects in the Polish potato processing plant. These findings therefore suggest another mechanism than in our study. However, precipitins or specific IgG to potato were not reported in the quoted Polish study.

The occurrence of specific IgG antibodies directed against other antigens than potato-derived proteins cannot be excluded but is not likely since the $IgG_{(4)}$ response was completely inhibited with potato extract. It can be hypothesised that an IgG response to fungi was not part of the observed response to airborne dust because this mixture did not contain enough fungal antigens. A suggestion for further study is to measure specific IgG and/or precipitins to fungal antigens in serum samples.

In contrast with our study, positive associations between specific IgG antibodies and respiratory symptoms, suggesting Hypersensitivity Pneumonitis, have been observed in other occupational settings.[21-24] However, the role of the IgG_4 subclass has not yet been elucidated.[25] It has been suggested that IgG_4 may have a protective effect as 'blocking antibody'.[20]

Acute respiratory health indices and relationships with exposure

The rationale of this study was the occurrence of work-related respiratory health complaints among potato processing workers. The first aim was to quantify the occurrence of work-related respiratory symptoms among workers of all four facilities. Sixteen percent of the workers reported one or more acute symptoms as 'occurring during work more often than normal' (cough, phlegm, wheezing, shortness of breath and chest tightness). The prevalence was highest in plant 2 (43%) where initially the most severe work-related complaints had been reported. The prevalence of chronic respiratory symptoms was more or less similar in all four plants.

In Chapter 6 it was found that a higher prevalence of work-related obstructive symptoms was associated with a higher endotoxin exposure. However, in the sub-study in one of the plants described in Chapter 5, these symptoms seemed to occur mainly in the low endotoxin exposure group. It can be hypothesised that endotoxin exposure in this plant was overall too low to cause respiratory symptoms, and the reported symptoms were for the greater part due to exposure to other dust constituents or chemicals. A drawback of our method to assess acute work-related respiratory symptoms was that a self-administered questionnaire was completed only once at the beginning of the processing campaign, possibly leading to recall-bias. In the peak flow diary (see Chapter 6), workers could indicate whether they suffered from respiratory symptoms during work, for a three-week period. Although peak flow records were kept regularly, symptoms were only occasionally recorded in this diary.

Another aim of this study was to describe objective acute respiratory health indices, in order to study more subtle work-related respiratory effects, not necessarily leading to the clinical manifestation of symptoms. Indices used in this study were lung function changes across the work shift. In many studies, across-shift lung function changes have been measured to assess acute respiratory health outcome, most frequently using repeated spirometry.[26–28] In Chapter 5, spirometric lung function changes across the afternoon shift (3–11 p.m.) were described. Repeated spirometry is a time-consuming technique which always requires supervision. Therefore, measurements on other work shifts, and on exposure-free days were not feasible. The given disadvantages do not hold for self-monitoring of peak flow. In Chapter 6, acute changes in peak flow

across different types of work shifts and across leisure days were described. The used mini-Wrights peak flow meters are easy to carry and use, and with a relatively small effort many lung function measurements over time could be performed. This was, however, done without supervision, and thus quality control was not possible. Moreover, the precision of spirometric lung function measurements is higher when compared with peak flow measurements. An advantage of peak flow measurements appeared to be the absence of an obvious learning effect, opposite to repeated spirometric lung function measurements. The occurrence of a learning effect has been described in a study using the same type of peak flow meters among adults[29], but was only apparent during the first day of the measurement period.

Across-shift lung function changes are also influenced by a circadian rhythm. Thus, dependent on the time of the day, an eight-hour lung function change can be positive or negative, independent of external influences. Differences in peak flow change between work days and leisure days are possibly indicative of work-related respiratory effects. Comparisons were difficult to make since across-shift peak flow change was highly influenced by timing of work shift. In our study, peak flow changes on leisure days were measured at similar times as the afternoon shift. Comparisons between Afternoon shift and leisure days (Table 6.3) revealed no apparent differences in peak flow change, indicating that overall no work-related lung function effects were present during the Afternoon shift.

In a number of studies on respiratory health, repeated peak flow measurements have been used to calculate an index of daily peak flow variability, such as the Amplitude/Mean ratio (AMP/MEAN). Descriptive statistics of this index in Table 6.2 showed different patterns for different work shifts; the level of variability was largely influenced by measurement times. The highest levels of AMP/MEAN were observed for the morning shift; probably due to the early morning (6 – 7 a.m.) peak flow measurement, which is close to the minimum of the circadian rhythm. A straightforward analysis of peak flow at the start and at the end of the work shift seemed to provide a more valid outcome variable, because the timing of measurements absent from work differed between workers.

Non-specific airway inflammation comprised the third hypothesised mechanism. Endotoxin exposure levels were in the range where acute effects have been reported.[26-28,30-32] Relationships between across-shift lung function changes and endotoxin exposure were investigated in order to evaluate whether endotoxin played a role in respiratory effects in this industry. Both spirometric lung function change (PEF and FEV_1) and change in peak flow determined with mini-Wright meters were related to endotoxin exposure. In Chapter 5, lung function decrease across the afternoon shift in workers exposed to endotoxin concentrations of on average 56 EU/m^3 was larger than in workers exposed to low endotoxin exposures of on average 21 EU/m^3. A no-effect level for airborne endotoxin exposure has been suggested to be 50 EU/m^3.[33] The largest differences between these groups were observed on the first work day after a 3-day absence from work; 3% for the difference in ΔFEV_1 and 11% for the difference in ΔPEF. On the second and third day, smaller and nonsignificant differences in across-shift lung function change between the two groups were found. This can be caused by a short-term tolerance to endotoxin.[34] In our study, another plausible explanation was that workers did not recover from the large lung function decrease across the first day, indicated by a lower pre-shift lung function on the second day as compared to pre-shift lung function on the first day. The decrease on the first day among highly exposed workers amounted to 5% in FEV_1 and 8% in PEF, which was 3–5% larger than expected on the basis of the circadian rhythm.

In Chapter 6, a significant relationship between endotoxin exposure and peak flow change across the afternoon shift was observed. Coefficients derived from linear regression models for peak flow change across the morning and the night shift, were not significantly different from the coefficient obtained from models using data of the afternoon shift. A multiple linear regression model with adjustment for shift was therefore applicable to describe endotoxin-related peak flow changes for all types of work shifts. Over the range of endotoxin exposure in this study (53 to 8,167 EU/m^3), a mean across-shift peak flow decrease of 3% could be estimated. Also in these analyses, endotoxin-related peak flow change was steeper on the first day after a few days absent from work; over the range in endotoxin exposure a 5% PEF decrease across the first day was estimated.

Findings in Chapters 5 and 6 strongly suggest a role of endotoxin in the etiology of acute work-related respiratory effects. Mean lung function decrease

was relatively small and seemed as such of little clinical importance. However, it is possible that some individuals experienced large lung function drops (>10%) during the work shift, which might be accompanied by respiratory symptoms. Results in our study suggest a predominant role of a non-specific airway inflammation. On some occasions in this industry, malaise and muscular pains have been reported (see Chapter 1); mainly in plant 2. This agrees with similar effects of high endotoxin exposure described in the literature.[35] A drawback of our study was that physiological markers of (non-specific) inflammation were not measured. A possible approach would have been to draw blood samples before and after work, and determine leucocytes, acute-phase proteins or other markers for inflammation.

It can be expected that endotoxin is not the only microbial component that has caused non-specific inflammatory reactions in our study. Endotoxin concentrations were significantly related to total bacterial counts (Table 2.2). Therefore, other bacteria-derived constituents could have played a role too. Peptidoglycan is another bacterial component that might induce inflammation after inhalation.[34,36] The absence of significant correlations between endotoxin concentrations and fungal counts indicate that it is unlikely that fungal components play a predominant role in the observed lung function changes. Apart from a Type III response to fungal antigens (see above), a non-specific inflammation due to fungal components could be hypothesised. Recent interest is for ß(1→3)-Glucans.[37-40], which can derive from fungi or from cereal and other plant materials. However, analysis of potato extract and potato starch extract from this industry revealed low glucan contents (<1 μg/mg) when compared to other vegetable products.[41]

Chronic respiratory effects

The endotoxin exposure levels found in this industry have been reported to be clearly associated with long-term lung function decreases in several other occupational settings.[30,42-47] In combination with the observation of endotoxin-related acute respiratory effects, the question arose whether the endotoxin levels were also related to chronic respiratory effects. In order to investigate effects of chronic exposures, a cross-sectional analysis was performed, with base-line lung function and the occurrence of chronic respiratory symptoms as health outcome variables of interest (Chapter 7).

Asthma symptoms in our study were more prevalent than among office workers, but similar as in other occupations exposed to organic dust. Bronchitis symptoms occurred less frequently than in other working populations. Lung function was on average higher than age- and height-specific predicted values. These external comparisons did not indicate chronic respiratory effects within the study group of potato processing workers. Furthermore, relationships between endotoxin exposure and respiratory health outcomes were not found. However, an important finding was that workers employed for more than five years showed a lower prevalence of respiratory symptoms, a better lung function and less atopy. This was suggestive of a health-related selection in this industry, resulting in the so-called Healthy Worker Effect (HWE). It can be speculated that symptomatic workers drop out of this industry within a few years. This can also be the case for 'campaign workers' who are exclusively employed in the processing season; if these workers develop work-related health disorders, it is possible that they will not return the next year.

The mentioned drop-out comprised one of the factors leading to a HWE. Another factor is pre-employment selection, making it less likely that individuals with health disorders start a job in this industry. A third possibility is a health-related job rotation within this industry. Indications for this phenomenon are the observation of lower endotoxin exposures in longer employed workers, and a higher prevalence of bronchitis symptoms in workers exposed to low dust levels. An analytical approach to minimise the influence of the HWE is to consider exposure-response relationships in various time periods since first exposure.[48] This was done using two groups of workers with a cut-off point of 12 years of employment (median). In general, exposure-response relationships tended to be different for workers employed for long and short periods, respectively, with stronger suggestions of adverse effects in the latter group. However, no significant exposure-response relationships could be detected in the group of shortly employed workers. It could be argued that in this situation a cross-sectional design was not optimal for detection of chronic effects. Furthermore, numbers of workers were relatively low in the applied analyses. A better approach would be a longitudinal design, following workers from the start of employment for a period of 5 – 10 years. In conclusion, no indications for chronic respiratory effects were found, which may be partially due to a health-related selection.

Moreover, statistical power was low to detect significant effects in the used study design.

General implications

Adverse effects of occupational endotoxin exposure

There is recent interest in setting an occupational exposure standard for endotoxin. A suggestion for a no-effect level is 50 EU/m^3.[33] In experimental (challenge) studies, concentrations below 100 EU/m^3 have been shown to be able to cause a detectable acute respiratory effect.[49] To the best of our knowledge, only in one field study[31] thus far lung function change was related to endotoxin levels in the range of 40 to 150 EU/m^3. Our findings described in Chapter 5 agree closely with the quoted study.

Little is known about the influence of host factors on the relationship between occupational endotoxin exposure and (acute) respiratory effects. For occupational health practice, it is useful to identify workers who may be more susceptible to endotoxin. In this thesis, an attempt was made to study whether smoking and atopy were modifying host factors in the studied relationships. Results in Table 6.4 indicate that no apparent differences in endotoxin exposure-response relationships existed between smokers and non-smokers, or between atopics and non-atopics. In the analyses presented in Table 5.4, numbers were too small to detect significantly different exposure-response relationships between smokers and non-smokers.

Furthermore, because of expected shifts in the circadian rhythm, it would be possible that workers react to a different extent to endotoxin for different times of the day. Analyses in Chapter 6 showed that exposure-response relationships seemed to be steepest during the Afternoon shift, but significant differences with Morning or Night shift were not found. An important implication of our findings is that if not all work shifts had been included in analyses, different conclusions with regard to the role of the host factors smoking and atopy could have been drawn. If only the Morning shift would have been studied, it was concluded that smokers showed larger endotoxin-related effects than non-smokers, while on the contrary on the Night shift non-smokers showed a steeper endotoxin exposure-response relationship. Thus, studies providing results for

only one of the work shifts should be interpreted with caution with regard to influencing effects of potential effect modifiers.

In cross-sectional studies on possible chronic effects of occupational endotoxin exposure, a Healthy Worker Effect is likely be present. To investigate the interfering role, exposure-response relationships in various time periods since first exposure can be considered. In addition, exposure levels in these various groups can also give information on health-related selections.

Prevention

In this study it has been suggested that high endotoxin exposure levels had an important etiological role in work-related respiratory effects. This raises the question what the sources of endotoxin exposure in this industry are, and which control measures can be applied in order to decrease exposure levels. In addition, dust levels were too high in some of the departments, independently of endotoxin exposure.

In order to decrease endotoxin exposure levels, the growth of (Gram-negative) bacteria should be inhibited. In Chapter 2 it has been suggested that airborne levels of both bacteria and endotoxin are dependent on process water temperature in the fibre dehydration machinery. This suggests that water cooling might inhibit bacterial growth. Other technical solutions would include radiation, which is probably too expensive to perform on such a large scale. The use of biocides would introduce a new occupational hygiene problem and, moreover, it is not applicable since (animal) food products are manufactured in this industry.

If it is not possible to reduce the source, isolation of the source should be considered.[50] For the process of fibre dehydration, closed centrifuges are used in three of the four plants. Stationary measurements showed that airborne levels of bacteria and endotoxin were 100-fold lower than in the fibre dehydration department of the plant with the open rolling mills. If replacement of the latter machinery with closed centrifuges is not feasible, a change of the ventilation system might be a solution. The present ventilation system consists of air showers, blowing fresh air into the area around the machines, and carry contaminated air out of the hall. However, workers move around and between the rolling mills, which means that an air shower is not the optimal solution in this department. Moreover, the front door is open during warm days, and due to uncontrolled air currents from outdoors, the contaminated air reaches the breathing zone of the

workers. To diminish the exposure in the control room next to the fibre dehydration department, an overpressure system could be applied in this room in order to separate the highly contaminated and clean working areas. Finally, as a temporary solution for some working tasks during which high exposures can be expected, such as cleaning and maintenance work, personal protective equipment can be advised. Unpublished work showed that the use of a half mask with P3 filter (CEN-standard: NEN-EN143) probably provides a reasonable protection to high endotoxin exposures, on condition that filter pieces are regularly replaced.

In the washing and grinding departments of one plant, a follow up study was performed. Maximum endotoxin exposure levels amounted to 5000 EU/m^3 (washing) and 1000 EU/m^3 (grinding). These numbers were clearly higher than in similar departments in the other three facilities (Table 2.5). During a rebuilding of the main wash, sieves were covered, spraying angle of the sprinkles was changed, the distance between main wash and grinding mill was enlarged and the open connection between these two departments was closed. To evaluate the effectiveness of these measures, and to investigate the influence of different water spraying techniques on the unchanged prewash, a follow-up study was performed in the three mentioned departments. It appeared that personal endotoxin exposure —in the washing as well as in the grinding department— had decreased with ±80%. Airborne concentrations of Gram-negative bacteria and endotoxin had decreased with 60–90% on main wash and grinding mill, and with 5–40% on the prewash. Three different types of spraying water were evaluated, and it appeared that level of bacterial water contamination (both viable bacteria and endotoxin) was very low. After this water had come in contact with the potatoes, contamination of the water increased drastically. Thus, an important source of Gram-negative bacteria and endotoxin on the prewash consisted of soil particles carried with the potatoes.

Problems with regard to 'nuisance' dust appeared mainly on drying, sacking and other expedition departments, and silos. In an unpublished follow-up study it was investigated which factors influenced exposure to starch dust and protein dust. Results showed that cleaning work was an important factor, mainly the use of compressed air increased dust exposure. It had been noticed that generally recommended industrial vacuum cleaners were used, but inappropriate

filters sometimes lead to the opposite of the desired control. In starch and protein sacking departments, filled bags fell down the conveyer belt generating a dust cloud. Finally, good housekeeping in a number of departments was not applied. Large amounts of dust were present on the floor surface, due to infrequent cleaning procedures. It can be expected that resuspension of this settled dust contributes to high personal dust exposures.

Conclusions and suggestions for further study

Conclusions

- Work-related acute respiratory effects in potato processing workers are most likely caused by high exposures to bacterial endotoxin.
- Allergic reactions to airborne dust are unlikely to play a predominant role in the etiology of acute respiratory effects.
- A strong humoral immune response to airborne dust is present, which is predominated by specific IgG_4 antibodies to potato proteins.
- In studies of acute respiratory effects among shift workers, it is important to account for the circadian rhythm of the lung function.
- An investigation of possible chronic effects of occupational exposures in the potato processing industry is hampered by the presence of health-related selection processes.
- Recycling of process water contributes to bacterial growth and hence to the build-up of endotoxin.
- Exposure to inhalable nuisance dust is too high in some departments.

Suggestions for further study

- Specific IgG and/or precipitins to fungal and bacterial compounds can be determined in workers' sera in order to exclude a possible Type III allergy against microbial antigens.
- Measurements of inflammatory markers in nasal lavage fluid or induced sputum before and after work can be performed to confirm the occurrence of airway inflammation.

- A longitudinal study among potato processing workers can be conducted in order to study chronic respiratory effects properly, and to evaluate health-related job-rotation and drop-out of workers.
- Control measures to reduce endotoxin exposure should be evaluated, with a priority for elimination and/or reduction at the source. Technical process changes seem necessary to achieve a sufficient decrease in endotoxin exposure.
- Control measures to reduce dust exposure should be applied, principally on the level of the process and the working environment.

References

1. Hollander A, Heederik D, Kauffman H. Acute respiratory effects in the potato processing industry due to a bio-aerosol exposure. *Occup Environ Med* 1994; 51: 73–78.
2. Dutkiewicz J. Bacteria, fungi and endotoxin as potential agents of occupational hazard in a potato processing plant. *Am J Ind Med* 1994; 25: 43–46.
3. Grimm HJ. Optical method for monitoring and characterisation of fine particles in workplace and urban environments. Occupational Hygiene '96. Promoting a healthy working environment. The British Occupational Hygiene Society, 1996; 63.
4. Jacobs RR. Endotoxins in the environment. *Int J Occup Environ Health* 1997; 3: S3–S5.
5. Eduard W. Exposure to non-infectious microorganisms and endotoxins in agriculture. *Ann Agric Environ Med* 1997; 4: 179–186.
6. Douwes J, Versloot P, Hollander A, Heederik D, Doekes G. The influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol* 1995; 61: 1763–1769.
7. Milton DK, Walters MD, Hammond K, Evans JS. Worker exposure to endotoxin, phenolic compounds, and formaldehyde in a fiberglass insulation manufacturing plant. *Am Ind Hyg Assoc J* 1996; 57: 889–896.
8. Sigsgaard T, Abell A, Jensen LD, Nielsen BH. Work-related symptoms and lung function measurements in paper mill workers exposed to recycled water. *Occup Hyg* 1994; 1: 177–189.
9. Forster HW, Crook B, Platts BW, Lacey J, Topping MD. Investigation of organic aerosols generated during sugar beet slicing. *Am Ind Hyg Assoc J* 1989; 50: 44–50.
10. Heederik D, Burdorf L, Boleij JSM, Willems H, Van Bilsen J. Pulmonary function and intradermal tests in workers exposed to soft-paper dust. *Am J Ind Med* 1987; 11: 637–645.
11. Heederik D, Boleij JSM, Kromhout H, Smid T. Use and analysis of exposure monitoring data in occupational epidemiology: An example of an epidemiological study in the Dutch animal food industry. *Appl Occup Environ Hyg* 1991; 6: 458–464.

12. Kromhout H, Tielemans E, Preller L, Heederik D. Estimates of indiviudal dose from current measurements of exposure. *Occup Hyg* 1996; 3: 23–39.
13. Rappaport SM. Assessment of long-term exposures to toxic substances in air. *Ann Occup Hyg* 1991; 35: 61–121.
14. Buringh E, Lanting R. Exposure variability in the workplace: Its implications for the assessment of compliance. *Am Ind Hyg Assoc J* 1991; 52: 6–13.
15. Francis M, Selvin S, Spear R, Rappaport S. The effect of autocorrelation on the estimation of workers' daily exposures. *Am Ind Hyg Assoc J* 1989; 50: 37–43.
16. Calkhoven PG, Aalbers M, Koshte VL, Griffioen RW, van Nierop JC, van der Heide D, Aalberse RC. Relationship between IgG_1 and IgG_4 antibodies to foods and the development of IgE antibodies to inhalant allergens. I. Establishment of a scoring system for the overall food responsiveness and its application to 213 unselected children. *Clin Exp Allergy* 1991; 21: 91–98.
17. Castells MC, Pascual C, Martin Esteban M, Ojeda JA. Allergy to white potato. *J Allergy Clin Immunol* 1986; 78: 1110–1114.
18. Bircher AJ, Van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. *Clin Exp Allergy* 1994; 24: 367–374.
19. Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, Kraft D, Scheiner O. Identification of allegens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens *Bet v* 1 and *Bet v* 2 (birch profilin). *J Allergy Clin Immunol* 1995; 95: 962–969.
20. Van der Zee JS, Aalberse RC. The role of IgG. In: Lessof MH, Lee TH, Kemeny DM (eds). Allergy: An international textbook. Chichester: John Wiley & Sons, 1987: 49–67.
21. Kurup VP, Barboriak JJ, Fink JN. Hypersensitivity Pneumonitis. In: Al-Doory Y, Domsan JF (eds). Mould Allergy. Philadelphia: Lea & Febiger, 1984: 216–243.
22. Christiani DC. Organic dust exposure and chronic airway disease. *Am J Respir Crit Care Med* 1996; 154: 833–834.
23. Eduard W, Sandven P, Levy F. Exposure and IgG antibodies to mould spores in wood trimmers: Exposure-response relationships with respiratory symptoms. *Appl Occup Environ Hyg* 1994; 9: 44–48.
24. Nielsen J, Welinder H, Schütz A, Skerfving S. Specific serum antibodies against phtalic anhydride in occupationally exposed subjects. *J Allergy Clin Immunol* 1988; 82: 126–133.
25. Aalberse RC, Van Milligen F, KY Tan, Stapel SO. Allergen-specific IgG_4 in atopic disease. *Allergy* 1993; 48: 559–569.
26. Donham K, Haglind P, Peterson Y, Rylander R, Belin L. Environmental and health studies of farm workers in Swedish confinement buildings. *Br J Ind Med* 1989; 46: 31–37.
27. Smid T, Heederik D, Houba R, Quanjer PhH. Dust- and endotoxin related acute lung function changes and work-related symptoms in workers in the animal feed industry. *Am J Ind Med* 1994; 25: 877–888.
28. Sigsgaard T, Malmros P, Nersting L, Petersen C. Respiratory disorders and atopy in Danish refuse workers. *Am J Respir Crit Care Med* 1994; 149: 1407–1412.
29. Boezen HM, Schouten JP, Postma DS, Rijcken B. Distribution of peak expiratory flow variability by age, gender and smoking habits in a random population sample aged 20–70 years. *Eur Respir J* 1994; 7: 1814–1820.

30. Sigsgaard T, Pedersen OF, Juul S, Gravesen S. Respiratory disorders and atopy in cotton, wool and other textile mill workers in Denmark. *Am J Ind Med* 1992; 22: 163 – 184.
31. Milton DK, Wypij D, Kriebel D, Walters MD, Hammond K, Evans JS. Endotoxin exposure-response in a fiberglass manufacturing facility. *Am J Ind Med* 1996; 29: 3 – 13.
32. Milton DK, Amsel J, Reed CE, Enright PL, Brown LR, Aughenbaugh GL, Morey PhR. Cross-sectional follow-up of a flu-like respiratory illness among fiberglass manufacturing employees: Endotoxin exposure associated with two distinct sequelae. *Am J Ind Med* 1995; 28: 469 – 488.
33. Heederik D, Douwes J. Towards and occupational exposure limit for endotoxins? *Ann Agric Environ Med* 1997; 4: 17 – 19.
34. Verhoef J, Kalter E. Endotoxic effects of peptidoglycan. In: ten Cate JW, Büller HR, Sturk A (eds). Bacterial endotoxins: Structure, biomedical significance, and detection with the Limulus Amebocyte Lysate test. New York, Alan R. Liss Inc, 1985: 101 – 112.
35. Rylander R. Evaluation of the risks of endotoxin exposures. *Int J Occup Environ Health* 1997; 3: S32 – S36.
36. Burroughs M, Rozdzinski E, Geelen S, Tuomanen E. A structure-activity relationship for induction of meningeal inflammation by muramyl peptides. *J Clin Invest* 1993; 92: 297 – 302.
37. Williams DL. (1→3)-ß-D-glucans. In: Rylander R, Jacobs R (eds). Organic dusts: exposure, effects, and prevention. Chicago: Lewis Publishers, 1994: 83 – 85.
38. De Lucca AJ, Brogden KA, French AD. Agglutination of lung surfactant with glucan. *Br J Ind Med* 1992; 49: 755 – 760.
39. Fogelmark B, Sjöstrand M, Rylander R. Pulmonary inflammation induced by repeated inhalations of ß(1→3)-D-glucan and endotoxin. *Int J Exp Pathol* 1994; 75: 85 – 90.
40. Rylander R, Persson K, Goto H, Yuasa K, Tanaka S. Airborne beta-1,3-glucan may be related to symptoms in sick buildings. *Indoor Environ* 1992; 1: 263 – 267.
41. Douwes J, Doekes G, Montijn R, Heederik D, Brunekreef B. Measurement of ß(1→3)-Glucans in occupational and home environments with an inhibition enzyme immunoassay. *Appl Environ Microbiol* 1996; 62: 3176 – 3182.
42. Kennedy SM, Christiani DC, Eisen EA, Wegman DH, Greaves IA, Olenchock SA, Ting-Ting Y, Pei-Lian L. Cotton dust and endotoxin exposure-response relationships in cotton textile workers. *Am Rev Respir Dis* 1987;135:194 – 200.
43. Heederik D, Brouwer R, Biersteker K, Boleij JSM. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. *Int Arch Occup Environ Health* 1991; 62: 595 – 601.
44. Smid T, Heederik D, Houba R, Quanjer PhH. Dust- and endotoxin-related respiratory effects in the animal feed industry. *Am Rev Respir Dis* 1992; 146: 1474 – 1479.
45. Zejda JE, Barber E, Dosman JA, Olenchock SA, McDuffie HH, Rhodes DVM, Hurst T. Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. *J Occup Med* 1994; 36: 49 – 56.
46. Preller L, Heederik D, Boleij JSM, Vogelzang PFJ, Tielen MJM. Lung function and chronic respiratory symptoms of pig farmers: focus on exposure to endotoxins and ammonia and use of disinfectants. *Occup Environ Med* 1995; 52: 654 – 660.

47. Schwartz DA, Donham KJ, Olenchock SA, Popendorf WJ, Scott van Fossen D, Burmeister LF, Merchant JA. Determinants of longitudinal changes in spirometric function among swine confinement operators. *Am J Respir Crit Care Med* 1995; 151: 47–53.
48. Choi BCK. Definition, sources, magnitude, effect modifiers, and strategies of reduction of the healthy worker effect. *J Occup Med* 1992; 10: 979–988.
49. Castellan RM, Olenchock SA, Kinsley KB, Hankinson JL. Inhaled endotoxin and decreased spirometric values. An exposure-response relation for cotton dust. *N Engl J Med* 1987; 317: 605–610.
50. Boleij JSM, Buringh E, Heederik D, Kromhout H. Occupational hygiene of chemical and biological agents. Amsterdam: Elsevier Science B.V., 1995: 225–255.

Glossary

AM	Arithmetic Mean
BCB	Bicarbonate Buffer
BTPS	Body Temperature and Pressure Saturated with water vapour
CFU	Colony Forming Units
CI	Confidence Interval
CV	Coefficient of Variation
D_{50}	50% cut-off diameter
DF	Degrees of Freedom
EIA	Enzyme Immuno Assay
ERS	European Respiratory Society
EU	Endotoxin Units
FEV_1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
GM	Geometric Mean
GSD	Geometric Standard Deviation
IgE	Immunoglobulin class E
IgG	Immunoglobulin class G
JEM	Job Exposure Matrix
κ	Cohen's *kappa* statistic for agreement of categorical data
LAL	Limulus Amoebocyte Lysate
ln	Natural logarithm
λ	Ratio of within- to between-worker variance in log-tranformed exposure
MMEF	Maximal Mid-Expiratory Flow
MS	Mean Squares
NOEL	No Observed Effect Level
OD	Optical Density
OES	Occupational Exposure Standard
PAS 6	Personal Air Sampler with an air inlet diameter of 6 mm
PBS	Phosphate Buffered Saline
PBTG	Phosphate Buffered Saline containing Tween-20 and Gelatin
PEF	Peak Expiratory Flow
PRR	Prevalence Rate Ratio
R	Correlation coefficient
$\hat{R}_{.95}$	Ratio of 97.5th and 2.5th percentiles of exposure distribution
RAST	Radio-AllergoSorbent Test
RAU	Relative Antigen Units
SD	Standard Deviation
SE	Standard Error
SS	Sum of Squares

Summary

Work-related respiratory health complaints have been reported in a potato processing plant. Responsible agents and the underlying mechanism for these disorders were unclear. On the basis of a preliminary survey and a literature study, three possible mechanisms could be suggested; a Type I allergy to potato, a Type III allergy to microbial components or potato (Hypersensitivity Pneumonitis), or non-specific airway inflammation caused by microbial components such as bacterial endotoxin. Aims of this study were to quantify the occurrence of work-related respiratory health effects in workers of all four plants of the potato processing company, and to study the three possible mechanisms.

Exposure to organic dust components is described in **Chapter 2.** Levels of inhalable nuisance dust were high (>10 mg/m^3) in some departments, where workers were involved in handling dried starch or protein. Levels of bacteria were overall high; in many departments above 10^5 CFU/m^3. Endotoxin levels were positively correlated with counts of Gram-negative bacteria. Endotoxin exposure differed strongly between plants and between job categories, but was overall very high; 23% of the workers had a mean endotoxin exposure above 1000 EU/m^3. Levels of airborne fungi were not extremely high; counts ranged from 200 to 15,000 CFU/m^3.

Chapter 3 describes an experimental study of filter elution methods for measurement of airborne potato antigens. The effect of different materials and methods for filter elution on potato antigen yield was studied using parallel airborne dust samples from a potato processing plant. Sonication plus vortexing showed on average a 13% higher yield than gentle shaking, and addition of 0.5% Tween-20 resulted in a mean increase of 11%. Potato antigens are proteins that are highly soluble in water, and can be readily eluted from airborne dust sampled on filters. Used materials and elution conditions have little effect on elution efficiency.

In **Chapter 4**, specific IgG and IgE response against airborne dust is described, in order to study the possible role of a Type I and a Type III allergy in the etiology of work-related respiratory effects. Specific IgG was detected in sera of nearly all 131 workers, while the IgG_4 subclass was found in about half of the workers. Specific $IgG_{(4)}$ was associated with airborne antigen exposure, and increased during the processing campaign. Control subjects with no history in potato processing work, and newly hired workers had low specific IgG levels

and no detectable specific IgG_4. No association was found between specific IgG and the prevalence of work-related respiratory symptoms. Moreover, both the difference in IgG titres between the potato processing workers and the non-exposed control group, and the exposure-related increase in specific IgG titres seemed to be mainly due to specific antibodies of the IgG_4 subclass. On the basis of these findings it is improbable that a Type III allergy and with that Hypersensitivity Pneumonitis plays a predominant role in the etiology of work-related respiratory effects. Specific IgE to airborne dust extract could not be detected in workers' sera, and therefore a Type I allergy was also highly unlikely to play a role in the etiology of work-related respiratory effects.

In **Chapter 5**, acute lung function changes across the Afternoon shift (3 – 11 p.m.) among 61 workers in one of the plants are described, and relationships with endotoxin exposure are investigated. A mean across-shift decrease in spirometric lung function variables was observed, which could be expected on the basis of the circadian rhythm. The largest decreases were observed on the first day after a 3-day absence from work, and pre-shift function on the second day was lower than pre-shift function on the first day. Exposure to endotoxin was relatively low in this plant, but on the basis of job title, workers could be divided in a low (AM = 21 EU/m^3) and a high (AM = 56 EU/m^3) exposure category. Twenty-five percent of the workers had reported work-related respiratory symptoms. Workers exposed to high endotoxin levels showed a larger across-shift decrease in lung function than workers exposed to low endotoxin exposures, the effect being most pronounced on the first day after a 3-day absence from work. From this study it was suggested that endotoxin exposure was related to acute respiratory health outcome, and is therefore likely to play a role in the etiology of work-related respiratory health effects.

In **Chapter 6**, acute PEF changes across the three different types of work shifts among 97 workers are described, and relationships with endotoxin exposure are investigated. As could be expected because of the circadian rhythm, peak flow increased during the Morning shift, and decreased during the Afternoon and the Night shift. A higher endotoxin exposure was associated with a smaller PEF increase across the Morning shift and a larger PEF decrease across the Afternoon and the Night shift. The largest effects occurred during the Afternoon shift, and during the first day after a period absence from work. Ten percent of the workers had reported work-related obstructive symptoms, and a

higher prevalence of these symptoms was associated with a higher endotoxin exposure. This study confirms the suggestion from Chapter 5 that endotoxin is likely to play a role in the etiology of work-related respiratory health effects.

In **Chapter 7**, the occurrence of possible chronic respiratory effects of organic dust exposure is investigated in a cross-sectional study among 135 workers of the four plants. Health outcome variables of interest were base-line lung function and the prevalence of chronic respiratory symptoms. External comparisons revealed that prevalence of respiratory symptoms was not higher than the prevalence in other occupational populations, and lung function was on average better than predicted. Internal comparisons were made using groups of workers exposed to low and high levels of dust, endotoxin and antigens. Apparent relationships between exposure indices and respiratory health outcome were not found. Workers employed for more than five years showed a lower prevalence of respiratory symptoms, a better lung function and less atopy, which was suggestive of a health-related selection. After stratification for employment duration, no significant relationships were found either, but relationships tended to be different for workers employed for long and short periods, respectively, with more suggestions for adverse effects in the latter group. Thus, no chronic respiratory effects could be found in the used cross-sectional design, which may be partially due to a health-related selection.

In **Chapter 8**, main findings and answers to the research questions are discussed. Sixteen percent of the workers had indicated work-related respiratory symptoms. Both Type I or Type III allergy are unlikely to play a role in the occurrence of acute respiratory effects. Results in this thesis strongly suggest a predominant role of non-specific airway inflammation caused by bacterial endotoxin in the etiology of respiratory effects. Measures to control bacterial growth and with that endotoxin exposure are required. An important source of bacteria and endotoxin appeared to be the recycling of process water. Next to endotoxin, exposure to nuisance dust was too high in some of the departments, and should therefore be controlled as well. Finally, cross-sectional analyses did not show chronic respiratory effects in potato processing workers. A Healthy Worker Effect seemed present, which hampered a proper investigation of chronic effects in the current workforce.

Samenvatting

Werknemers van een aardappelverwerkende fabriek hadden klachten over longen en luchtwegen tijdens het werk. Het was niet duidelijk welke stoffen dit konden veroorzaken, en wat het onderliggende mechanisme was. Een oriënterend onderzoek en een literatuurstudie leverden drie mogelijke mechanismen op. Dit waren een Type I allergie tegen aardappel, een Type III allergie tegen microbiële componenten of aardappel (Allergische Alveolitis), en een aspecifieke ontstekingsreactie in de luchtwegen als gevolg van microbiële componenten zoals endotoxine. Doelstellingen van dit onderzoek waren het kwantificeren van het voorkomen van werkgerelateerde respiratoire aandoeningen bij werknemers in alle vier fabrieken van het bedrijf, en het bestuderen van de drie gesuggereerde mechanismen.

In **Hoofdstuk 2** wordt de blootstelling aan organisch stof beschreven. De concentratie inhaleerbaar hinderlijk stof was hoog (> 10 mg/m^3) op een aantal afdelingen waar met de gedroogde eindproducten zetmeel en eiwit werd gewerkt. De hoeveelheid bacteriën in de lucht was hoog; op veel afdelingen boven 10^5 KVE/m^3. Er was een positieve correlatie tussen de gehaltes endotoxine en Gram-negatieve bacteriën in de lucht. Endotoxineblootstelling verschilde sterk tussen de fabrieken en tussen de functies, maar was in het algemeen zeer hoog; 23% van de werknemers had een gemiddelde endotoxine blootstelling boven 1000 EU/m^3. De hoeveelheid schimmels in de lucht was niet extreem hoog; concentraties varieerden van 200 tot 15.000 KVE/m^3.

In **Hoofdstuk 3** wordt een experimentele studie beschreven naar extractiemethoden voor stoffilters om aardappelantigenen in de lucht te meten. De invloed van verschillende materialen en extractiemethoden werd onderzocht met behulp van parallelle stofmonsters in één van de fabrieken. Ultrasoon trillen in combinatie met vortexen gaf een 13% hogere antigeenopbrengst dan alleen schudden, terwijl het toevoegen van 0,5% Tween-20 in een toename van 11% resulteerde. Aardappelantigenen zijn eiwitten die goed in water oplossen, en zijn gemakkelijk te extraheren uit stof dat op filters is verzameld. De materialen en methoden van extractie hebben weinig effect op het rendement van de extractie.

In **Hoofdstuk 4** wordt de specifieke IgG en IgE respons tegen stof uit de lucht binnen de fabrieken beschreven, om het optreden van een Type I en een Type III allergie te onderzoeken. Specifiek IgG was detecteerbaar in het serum van bijna alle 131 werknemers, terwijl specifieke antilichamen van de IgG_4 subklasse in

serum van ongeveer de helft van de werknemers kon worden aangetoond. Specifiek $IgG_{(4)}$ was gerelateerd aan de persoonlijke antigeenblootstelling, en nam toe gedurende de campagne. In serum van controles die nooit in aardappelverwerking hadden gewerkt, en van nieuwe werknemers waren de gehaltes specifiek IgG laag, en kon specifiek IgG_4 niet worden gedetecteerd. Er was geen verband tussen specifiek IgG en de prevalentie van werkgerelateerde klachten over longen en luchtwegen. Bovendien bleek zowel het verschil in IgG titers tussen aardappelwerkers en controles, als de stijging van de IgG titers tijdens de campagne, veroorzaakt te worden door de IgG_4 subklasse. Op grond van deze resultaten is het onwaarschijnlijk dat Type III allergie en daarmee Allergische Alveolitis een belangrijke rol speelt in de etiologie van de luchtwegaandoeningen. Specifiek IgE tegen stof uit de lucht in sera van de werknemers kon niet worden aangetoond, dus het was zeer onwaarschijnlijk dat een Type I allergie hier een belangrijk mechanisme vormde.

In **Hoofdstuk 5** worden acute longfunctieveranderingen tijdens de middagdienst (15.00 – 23.00) bij 61 werknemers van één van de fabrieken beschreven, en relaties daarvan met endotoxine blootstelling onderzocht. Spirometrische longfunctievariabelen vertoonden een gemiddelde daling over de werkdag, wat te verwachten was op basis van het circadiaan ritme. De grootste dalingen vonden plaats op de eerste werkdag na een vrije periode van drie dagen. De longfunctie aan het begin van de tweede werkdag was lager dan aan het begin van de eerste werkdag. Endotoxineblootstelling was relatief laag in deze fabriek, maar op basis van functie konden werknemers worden ingedeeld in een laag (AM = 21 EU/m^3) en een hoog (AM = 56 EU/m^3) blootgestelde groep. Een kwart van de werknemers had klachten over longen en luchtwegen tijdens het werk. Hoog blootgestelde werknemers vertoonden grotere dalingen in longfunctie dan laag blootgestelde werknemers. Dit verschil was het grootst op de eerste werkdag na drie vrije dagen. Resultaten van dit onderzoek suggereren dat endotoxineblootstelling is gerelateerd aan acute longfunctieveranderingen, en daarmee dat aspecifieke ontstekingsreacties door endotoxine waarschijnlijk een rol spelen in de etiologie van de luchtwegaandoeningen bij de werknemers.

In **Hoofdstuk 6** worden piekstroomveranderingen tijdens de verschillende typen diensten en vrije dagen bij 97 werknemers beschreven, en verbanden met endotoxine blootstelling onderzocht. Als gevolg van het circadiaan ritme, steeg de piekstroom tijdens de ochtenddienst en daalde de piekstroom tijdens de avond-

en nachtdienst. Een hogere endotoxineblootstelling ging samen met een kleinere stijging van de piekstroom over de ochtenddienst, en met een grotere daling van de piekstroom over de avond- en nachtdienst. De sterkste effecten traden op tijdens de middagdienst, en tijdens de eerste werkdag na een paar vrije dagen. Tien procent van de werknemers had klachten over longen en luchtwegen tijdens het werk, en een hogere prevalentie van deze symptomen ging samen met een hogere endotoxine blootstelling. Resultaten van dit onderzoek ondersteunen de suggestie uit Hoofdstuk 5 dat aspecifieke ontstekingsreacties door endotoxine waarschijnlijk een belangrijk mechanisme vormen bij het optreden van acute aandoeningen van longen en luchtwegen.

In **Hoofdstuk 7** worden mogelijke chronische effecten op longen en luchtwegen van organisch stof blootstelling bestudeerd in een dwarsdoorsnede onderzoek bij 135 werknemers uit de vier fabrieken. Effectparameters waren longfunctie aan het begin van de campagne, en het voorkomen van chronische respiratoire symptomen. Externe vergelijkingen lieten zien dat de prevalentie van symptomen vergelijkbaar was met die van andere beroepsgroepen, en dat de longfunctie gemiddeld hoger was dan voorspeld. Interne vergelijkingen werden gemaakt tussen groepen werknemers die laag en hoog waren blootgesteld aan stof, endotoxine, of antigenen. Consistente relaties tussen blootstellingsmaten en effectparameters werden niet gevonden. Werknemers die langer dan vijf jaar in deze industrie werkten, hadden minder respiratoire symptomen, een betere longfunctie, en minder atopie, hetgeen een selectie op basis van gezondheid suggereert. Na stratificatie voor aanstellingsduur waren ook geen consistente verbanden tussen blootstelling en effectparameters te zien in groepen werknemers met respectievelijk een lange en een korte aanstellingsduur. Echter, er waren verschillen tussen blootstellings-respons relaties tussen beide groepen, met meer suggesties voor negatieve effecten in de laatstgenoemde groep. Samengevat konden geen chronische respiratoire effecten worden aangetoond in dit dwarsdoorsnede onderzoek, hetgeen voor een deel te wijten kan zijn aan selectieprocessen in deze bedrijfstak.

In **Hoofdstuk 8** worden de belangrijkste bevindingen en antwoorden op de onderzoeksvragen bediscussieerd. Zestien procent van de werknemers had tijdens het werk wel eens klachten over longen en luchtwegen. Een allergie van zowel Type I als Type III speelt waarschijnlijk geen rol in het onderliggend mechanisme van de respiratoire effecten. Resultaten zoals beschreven in dit proef-

schrift geven sterke aanwijzingen voor een voorname rol van aspecifieke ontstekingsreacties in de luchtwegen door blootstelling aan endotoxine. Maatregelen om de hoge concentraties bacteriën en daarmee endotoxine te beheersen zijn noodzakelijk. Een belangrijke bron voor bacteriën en endotoxine bleek het hergebruiken van proceswater te zijn. Naast endotoxine was de blootstelling aan hinderlijk stof te hoog op een aantal afdelingen, en ook hier zijn beheersmaatregelen gewenst. Een dwarsdoorsnede onderzoek tenslotte leverde geen aanwijzingen voor chronische respiratoire effecten op. Er was waarschijnlijk een sterk 'Healthy Worker Effect' aanwezig, dat een passend onderzoek naar chronische effecten bij werknemers in de aardappelverwerkende industrie bemoeilijkt.

Appendix

Between- and within-observer agreement for expert judgement of peak flow graphs from a working population[1]

Abstract

Expert judgement of peak flow-time graphs provides an important tool to detect occupational asthma. This technique has mainly been used in clinics to evaluate variable airways obstruction and to assess potential work-related patterns. The reproducibility of this technique in an open working population is unknown. Agreement between and within nine experts was studied using peak flow-time graphs of 49 potato processing workers. All graphs were classified into four categories by the nine experts, while seven experts read ten graphs at two occasions. Thirty-four graphs (69%) were classified as 'no airways obstruction' while four graphs (8%) showed 'work-related airways obstruction'. There was only slight agreement between the nine experts; mean Cohen's kappa (κ) was 0.19. Agreement within experts was moderate; mean κ was 0.47 for judging graphs twice. Our results suggest that in a 'healthy' working population, judgement of peak flow graphs is not a favourable method for detection of airways obstruction. If this technique is applied in epidemiological studies, judgement of the graphs should be done by more than one expert.

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[1] Jan-Paul Zock, Daphnis Brederode, Dick Heederik.
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Introduction

Repeated peak flow measurements comprise the major method to determine acute variable airways obstruction.[1,2] This constitutes one of the methods for (early) detection of asthma and other respiratory disorders in occupational health practice. Methods and criteria for interpretation of peak flow data have been proposed by different investigators.[3-7] In general, distinction can be made between (1) calculation and evaluation of statistical indices and (2) plotting of peak flow versus time and judgement by one or more experts.

The calculation of statistical indices, typically a measure of peak flow variability such as Amplitude%Mean, is often used in epidemiological studies[3-6], although it is not clear whether the use of one single index provides a valid method to detect variable airways obstruction and further validation is required. In a population sample peak flow variability shows a wide distribution. Moreover, there is a large overlap between asthmatics and non-asthmatics.[3]

Expert judgement of peak flow graphs (classical visual method) is frequently used for screening in clinical studies[7] or occupational health surveys focusing on symptomatic workers[8,9]. The subjective character of this type of observations seems to be an obvious disadvantage. Venables and co-workers[10] judged peak flow-time graphs to determine work-related airways obstruction in a group of 61 workers exposed to isocyanate or acid anhydride. Complete agreement between the four observers was reached for 69% of the graphs, while agreement between at least three of the observers was reached for 97% of the graphs. Perrin *et al.*[7] found complete agreement among the three experts in 54 out of 61 judged graphs (89%) from workers exposed to various sensitising agents, with a history suggestive of occupational asthma. Liss and Tarlo[11] studied the level of agreement between two experts for judging of peak flow graphs of 31 workers visiting an occupational asthma clinic. They reported an agreement of 0.62, expressed as Cohen's kappa (κ). These three studies suggest a reasonable agreement between readers. However, the outcome of these clinical studies may not be representative for many occupational settings since a very high prevalence of asthmatic or other respiratory symptoms was found in these populations. The reproducibility of the expert judgement technique in open populations (e.g. industrial workers) is not clear. This study was performed to evaluate performance of the expert judgement technique for screening of work-related

airways obstruction within a large occupational health study in the potato processing industry.[12,13] In this industry, work-related symptoms have been reported and peak flow patterns suggesting work-related airways obstruction due to high organic dust exposures have been demonstrated. This paper focuses on between- and within-observer agreement for classification of peak flow graphs into four categories.

Methods

Population and peak flow measurements

In 1989 and 1992, occupational health surveys were conducted among potato processing workers.[9,13] Workers completed a short self-administered Dutch version of an internationally accepted respiratory questionnaire[14], supplemented with questions on work-related symptoms. Symptoms were considered to be work-related if they occurred during work 'more frequently than normal'.

Three work shifts were distinguished: Morning (7 a.m. – 3 p.m.), Afternoon (3 p.m. – 11 p.m.) and Night (11 p.m. – 7 a.m.). In one of the four plants shifts changed one hour earlier (6 a.m., 2 p.m., 10 p.m.). The sequence of shift rotation was Afternoon → Morning → Night → Afternoon, etcetera. A work period lasted alternately three or four days, with a subsequent leisure period of two days (after Morning and Afternoon shift) or three days (after Night shift).

Peak Expiratory Flow (PEF) was measured using mini-Wright peak flow meters three times (leisure days) or four times (work days) daily for a three-week period. On work days target times were (1) just after rising, before work; (2) in the middle of the work shift; (3) directly after the work shift and (4) before going to sleep. On leisure days target times were (1) just after rising; (2) in the middle of the day and (3) before going to sleep. Exact time of measurement was also recorded. On each occasion, three manoeuvres were performed[1,2] and recorded in the diary. Analyses were performed using the highest of the three manoeuvres. Peak flow-time graphs were made for each worker, and were inspected to detect obvious data errors. An example of a peak flow-time graph is presented in Fig. A.1. For each of the 23 days, the daily minimum, maximum and mean peak flow are shown. Furthermore, working days are indicated by shaded bars.

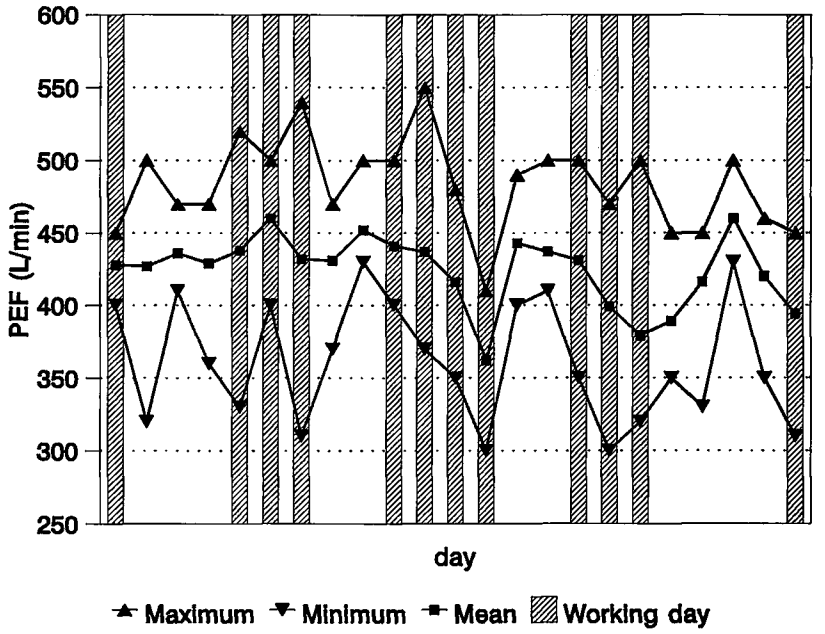

Fig. A.1: Example of a graph of peak flow (L/min) versus time, as handed to the experts

Expert judgement of peak flow-time graphs

Fifty peak flow-time graphs were selected for judgement by experts; 10 workers with a graph indicating possible airways obstruction according to the opinion of one of the authors, and an additional sample of 40, stratified by plant. This selection procedure was not influenced by information on any other study outcomes like age, symptoms or exposure. Nine experts in the field of respiratory health (3 physicians and 6 respiratory epidemiologists) classified each graph independently and blinded to other results into one of the following four categories based on a similar study[11]: (1) no airways obstruction; (2) airways obstruction, not work-related; (3) airways obstruction, work-related; (4) doubtful.

Two experts judged the 50 graphs in one session. The other seven experts read graphs in two sessions with an interval of at least five days. At each session, 30 graphs were observed. In order to study within-observer agreement, 10 graphs were evaluated at both occasions, without informing the experts. The first judgement of these graphs was used in analyses of between-observer agreement.

Statistical analyses

For each graph we calculated which category the majority of the nine experts selected. Both between- and within-observer agreement was expressed as Cohen's kappa (κ).[15] Between-observer agreement for classification of the graphs into one of the four categories was expressed in a κ that can be interpreted as an 'intraclass correlation coefficient'.[15] The mean κ is calculated as weighed average for the four categories as proposed by Landis and Koch.[16] The obtained results are comparable to the study of Liss and Tarlo[11] when categories (3) (airways obstruction, work-related) and (4) (doubtful) are joined. Within-observer agreement was determined using the 10 graphs judged twice by seven experts, yielding a κ for each of these seven observers.

Results

One of the 50 graphs was eliminated from statistical analyses; the time of peak flow measurement had not been consequently recorded, which made plotting of an appropriate graph impossible. In Table A.1 characteristics are given of the 49 male shift workers in this study. Five of the 49 workers had been examined in 1989, the other 44 in 1992.Work-related symptoms comprised cough, phlegm, shortness of breath and chest tightness. FEV_1 was on average higher than the age- and height-specific predicted value[17] (Student's paired t-test; $P=0.05$).

Table A.1: Characteristics of the study population ($N=49$)

Age: mean (range)	39 (22–58)
Current smokers: *n* (%)	16 (33%)
Respiratory symptoms*: *n* (%)	9 (18%)
Work-related symptoms†: *n* (%)	6 (12%)
FEV_1 ‡: mean (range)	103 (76–121)

* At least one symptom from standard questionnaire[14]
† At least one work-related respiratory symptom
‡ Percentage of age- and height-specific predicted value[17] ($n=38$)

Complete agreement between the nine experts was reached for 6 of the 49 graphs (12%); all concerning agreement on category (1): no airways obstruction. In 34 of the 49 graphs, the majority of the nine observers selected category (1). A majority for category (2) was reached for four graphs, and the same was true for categories (3) and (4). Two of the four workers (50%) who were considered as (3) 'airways obstruction, work-related' had indicated work-related symptoms in the questionnaire, against four of the 45 others (9%) (Fisher's Exact test; $P=0.07$). None of the four workers who were considered as (2) 'airways obstruction, not work-related' had indicated work-related symptoms, but 2 (50%) had indicated chronic respiratory symptoms.

Table A.2 shows the agreement between the nine observers for classification of 49 graphs into four categories, expressed as Cohen's kappa (κ). The standard error of κ was approximately 0.024 under the null hypothesis $\kappa=0$. The weighed average of κ for four categories was 0.19; 95% confidence interval (CI): 0.16 to 0.22. When categories (3) 'airways obstruction, work-related' and (4) 'doubtful' were combined, the weighed average of κ was 0.24; 95% CI: 0.20–0.28. The κ for within-observer agreement for judgement of 10 graphs twice, ranged from 0.35 to 0.58 with a mean of 0.47.

Table A.2: Agreement between classifications of nine observers on four or three categories, expressed as Cohen's kappa (κ)

	four categories		three categories	
Category	κ	95% CI*	κ	95% CI
1 No airways obstruction	0.26	0.22–0.31	0.28	0.23–0.32
2 Airways obstruction, not work-related	0.14	0.09–0.19	0.13	0.08–0.18
3 Airways obstruction, work-related	0.26	0.21–0.31	0.24	0.19–0.29
4 Doubtful	0.05	0.00–0.10		

* Confidence Interval

Discussion

In this study, reproducibility of expert judgement of peak flow graphs by different observers was evaluated. Agreement between observers was very poor, while the agreement within observers was reasonable. Classification by one single expert seems therefore not a favourable method to detect work-related airways obstruction.

The level of agreement between the classifications of the different experts was very low ($\bar{\kappa}=0,19$). A κ below 0.4 indicates that the agreement is mainly explained by coincidence.[15] The agreement for 'airways obstruction, work-related' ($\kappa=0.26$), however, was slightly better than for 'airways obstruction, not work-related' ($\kappa=0.14$). Liss and Tarlo[11] found an overall κ of 0.62 using the same categories as in our study, but joined the categories 'doubtful' and 'work-related'. Initially, we did not follow this procedure because the category 'doubtful' probably consisted of subjects for whom the diagnosis airways obstruction was difficult to make. For a fair comparison, we eventually joined the categories 3 and 4, resulting in an overall κ of 0.24, which is still low.

The most plausible explanation for low levels of agreement observed in our study is the low prevalence of airways obstruction; work-related airways obstruction was detected in 4/49=8% of the workers. In most other studies, subjects were attending a respiratory clinic, resulting in an occupational asthma

prevalence of 36%[11], 25%[10] and 41%[7]. It has been described previously that a low prevalence is associated with a low κ.[18,19] Another explanation may be the difference in experience/background. In many studies described in the literature, peak flow graphs were judged by the investigators themselves who generally have similar (e.g. clinical) backgrounds. In our study the observers received only basic criteria.

The within-observer κ was on average 0.47, indicating a moderate agreement between the two readings of the same graph by an observer. In combination with the poor between-observer agreement, we deduce that classification by one single expert probably does not provide a reliable index for airways obstruction in open populations. Our results indicate that whenever the classical visual method is used in occupational epidemiological studies, the classification of the graphs should be done by more than one single expert.

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References

1. Quanjer PH, Lebowitz MD, Gregg I (eds). Peak expiratory flow. Draft conclusions and recommendations of a Working Party of the European Respiratory Society. 1992.
2. Moscato G, Godnic-Cvar J, Maestrelli P, Malo J-L, Burge PS. Statement on self-monitoring of peak expiratory flows in the investigation of occupational asthma. *Eur Respir J* 1995; 8: 1605–1610.
3. Higgins BG, Britton JR, Chinn S, Jones TD, Jenkinson D, Burney PGJ, Tattersfield AE. The distribution of peak expiratory flow variability in a population sample. *Am Rev Respir Dis* 1989; 140: 1368–1372.
4. Quackenboss JJ, Lebowitz MD, Krzyzanowski M. The normal range of diurnal changes in peak expiratory flow rates. *Am Rev Respir Dis* 1991; 143: 323–330.
5. Boezen HM, Schouten JP, Postma DS, Rijcken B. Distribution of peak expiratory flow variability by age, gender and smoking habits in a random population sample aged 20–70 years. *Eur Respir J* 1994; 7: 1814–1820.
6. Hetzel MR, Clark TJH. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. *Thorax* 1980; 35: 732–738.

7. Perrin B, Lagier F, l'Archevêque J, Cartier A, Boulet L-P, Côté J, Malo J-L. Occupational asthma: validity of monitoring of peak expiratory flow rates and non-allergic bronchial responsiveness as compared to specific inhalation challenge. *Eur Respir J* 1992; 5: 40-48.
8. Blainey AD, Ollier S, Cundell D, Smith RE, Davies RJ. Occupational asthma in a hairdressing salon. *Thorax* 1986; 41: 42–50.
9. Hollander A, Heederik D, Kauffman H. Acute respiratory effects in the potato processing industry due to a bio-aerosol exposure. *Occup Environ Med* 1994; 51: 73–78.
10. Venables KM, Burge PS, Davison AG, Newman Taylor AJ. Peak flow rate records in surveys: Reproducibility of observers' reports. *Thorax* 1984; 39: 828–832.
11. Liss GM, Tarlo SM. Peak expiratory flow rates in possible occupational asthma. *Chest* 1991; 100: 63–69.
12. Zock JP, Heederik D, Kromhout H. Exposure to dust, endotoxin and micro-organisms in the potato processing industry. *Ann Occup Hyg* 1995; 39: 841-854.
13. Zock JP, Doekes G, Heederik D, van Zuylen M, Wielaard P. Airborne dust antigen exposure and specific IgG response in the potato processing industry. *Clin Exp Allergy* 1996; 26: 542–548.
14. Biersteker K, Dijk WH van, Eissens JBMF, Geuns HA van. Ervaringen met geneeskundig onderzoek op CARA bij gemeentepersoneel te Rotterdam in 1970 –1971. *T Soc Geneesk* 1974; 52: 158–162. (In Dutch, with a summary in English.)
15. Fleiss JL. Statistical methods for rates and proportions. New York: Wiley, 1981: 212–236.
16. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 150–174.
17. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernoult JC. Lung volumes and forced ventilatory flows. *Eur Respir J* 1993; 6, Suppl. 16: 5–40.
18. Lantz CA, Nebenzahl E. Behavior and interpretation of the κ statistic: Resolution of the two paradoxes. *J Clin Epidemiol* 1996; 49: 431–434.
19. Armstrong BK, White E, Saracci F. Principles of exposure assessment in epidemiology. Oxford: UP, 1992.

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Levensoverzicht

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